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ARTICLE

Low-Density Lipoprotein Cholesterol and the Risk of Cancer: A Mendelian Randomization Study

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Background Low plasma levels of low-density lipoprotein (LDL) cholesterol are associated with an increased risk of cancer, but whether this association is causal is unclear.

Methods We studied 10 613 participants in the Copenhagen City Heart Study (CCHS) and 59 566 participants in the Copenhagen General Population Study, 6816 of whom had developed cancer by May 2009. Individuals were genotyped for *PCSK9* R46L (rs11591147), *ABCG8* D19H (rs11887534), and *APOE* R112C (rs429358) and R158C (rs7412) polymorphisms, all of which are associated with lifelong reduced plasma LDL cholesterol levels. Plasma LDL cholesterol was calculated using the Friedewald equation in samples in which the triglyceride level was less than 354 mg/dL and measured directly by colorimetry for samples with higher triglyceride levels. Risk of cancer was estimated prospectively using Cox proportional hazards regression analyses and cross-sectionally by logistic regression analyses. Causality was studied using instrumental variable analysis. All statistical tests were two-sided.

Results In the CCHS, compared with plasma LDL cholesterol levels greater than the 66th percentile (>158 mg/dL), those lower than the 10th percentile (<87 mg/dL) were associated with a 43% increase (95% confidence interval [CI] = 15% to 79% increase) in the risk of cancer. The polymorphisms were associated with up to a 38% reduction (95% CI = 36% to 41% reduction) in LDL cholesterol levels but not with increased risk of cancer. The causal odds ratio for cancer for a 50% reduction in plasma LDL cholesterol level due to all the genotypes in both studies combined was 0.96 (95% CI = 0.87 to 1.05), whereas the hazard ratio of cancer for a 50% reduction in plasma LDL cholesterol level in the CCHS was 1.10 (95% CI = 1.01 to 1.21) (*P* for causal odds ratio vs observed hazard ratio = .03).

Conclusion Low plasma LDL cholesterol levels were robustly associated with an increased risk of cancer, but genetically decreased LDL cholesterol was not. This finding suggests that low LDL cholesterol levels per se do not cause cancer.

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In 1974, Rose et al. (1) observed that plasma cholesterol levels were lower than expected in men with colon cancer. Since then, several prospective studies (2–7) have confirmed an association between low plasma levels of cholesterol and an increased risk of cancer. The reason for this observation is unclear; theoretically, it could reflect a causal relationship (ie, low plasma low-density lipoprotein [LDL] cholesterol causes cancer) or be due to a confounder that causes both low plasma LDL cholesterol and cancer or to reverse causation (ie, a preclinical cancer that reduces plasma LDL cholesterol levels) (3,7). Whether low LDL cholesterol causes cancer is an important question. However, data from randomized intervention trials of LDL cholesterol lowering with statins conducted during the last three decades and several large meta-analyses that include data from more than 90 000 patients [summarized in (8)] have lessened this concern. Nevertheless, combined results from rodent studies (9), an early trial of the lipid-lowering agent clofibrate (10), and a recent lipid lowering trial [Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) Study (11)] have led to renewed concerns that lowering plasma cholesterol via pharmacological interventions might increase the risk of cancer.

Mendelian randomization is an epidemiological approach that can be used to study a potential causal relationship because it circumvents confounding and reverse causation (12–17). This approach makes use of the random assortment of genetic variants during gamete formation, which is analogous to the random assignment of patients to placebo or active treatment in a clinical intervention trial, to assess the causal relationship between a modifiable risk factor and disease. For example, with this approach, genetic variants that are associated with low plasma levels of LDL cholesterol would be largely unconfounded by other risk factors

for low plasma LDL cholesterol, such as sex, smoking history, body mass index (BMI), hypertension, and diabetes mellitus, and can therefore be used to assess the consequences of lifelong low plasma LDL cholesterol levels independent of other risk factors with no risk of reverse causation (14).

In this study, we tested the hypothesis that there is a robust and potentially causal association between low LDL cholesterol levels and an increased risk of cancer. We tested the robustness of the association by adjusting the risk estimates for age, sex, BMI, hypertension, diabetes mellitus, current smoking, and statin use; additional adjustments included ischemic heart disease, exclusion of events that occurred within 4 years after LDL cholesterol measurement, and exclusion of participants who were treated with statins. We tested the potential causality of the association by examining whether genotypes that are associated with lifelong reduced plasma LDL cholesterol levels were associated with an increased risk of cancer. For the latter analysis, we compared the association between genotypes and plasma LDL cholesterol levels with the association between genotypes and the risk of cancer, performed instrumental variable analysis to estimate the causal association between genetically reduced levels of LDL cholesterol and the risk of cancer, and compared this estimate with the corresponding estimate from conventional observational epidemiology.

CONTEXT AND CAVEATS

Prior knowledge

Low plasma levels of low-density lipoprotein (LDL) cholesterol are associated with an increased risk of cancer, but it is unclear if this association is causal.

Study design

A Mendelian randomization study that used instrumental variable analysis to examine whether single-nucleotide polymorphisms in *PCSK9*, *ABCG8*, and *APOE* that are associated with lifelong reduced plasma LDL cholesterol levels are causally linked to an increased risk of cancer among participants in two Danish general population studies.

Contribution

Low plasma LDL cholesterol levels were robustly associated with an increased risk of cancer, but genetically reduced LDL cholesterol levels (due to polymorphisms that are associated with lifelong reduced plasma LDL cholesterol levels) were not.

Implications

Low LDL cholesterol levels per se do not cause cancer.

Limitations

The genes included in the analysis explain only half of the total genetic variation in plasma LDL cholesterol level. All participants were white and of Danish descent; thus, the results may not apply to other races or ethnicities.

From the Editors

Participants and Methods

Participants

The study population consisted of participants in two studies in Denmark—the Copenhagen City Heart Study (CCHS) and the Copenhagen General Population Study (CGPS)—both of which were approved by the institutional review boards and Danish ethical committees (No. KF-V.100.2039/91, KF-01-144/01, H-KF-01-144/01) and conducted according to the Declaration of Helsinki. Written informed consent was obtained from participants. All participants were white and of Danish descent. None of the participants appeared in more than one study, which allowed us to combine the two similar general population studies to obtain maximal statistical power. Follow-up was 100% complete in both studies.

The CCHS is a prospective study of the general population of Copenhagen, Denmark, that was initiated in 1976–1978 with follow-up examinations in 1981–1983, 1991–1994, and 2001–2003 (18). Participants in the CCHS (n = 14 223) were randomly selected from the national Danish Civil Registration System to reflect the adult Danish population ranging from age 20 to 80 years or older. At each of the four examinations, participants completed a questionnaire, underwent a physical examination, and provided blood samples. Plasma was separated, and biochemical analyses were performed on the same day. Blood samples for DNA extraction and genotyping were available for 10 593 (99.8%) of the 10 613 participants for whom plasma LDL cholesterol measurements were available. Median follow-up time up to May 9, 2009, was 15.2 years (range = 4–17.6 years).

The CGPS (n = 59 566) is a study of the general population of Denmark that was initiated in 2003 with enrollment ongoing (19). The study is partly cross-sectional and partly prospective in that cancer endpoints were collected until May 9, 2009. Participants were selected and examined exactly as described for the CCHS. Blood samples for DNA extraction and genotyping were available for 56 624 (95.1%) of the 59 566 participants for whom plasma LDL cholesterol measurements were available.

Ascertainment of a Diagnosis of Cancer

Diagnoses of invasive cancer from 1947 to May 9, 2009, were obtained from the Danish Cancer Registry, which identifies 98% of all cancers diagnosed in Denmark (20,21), and from the national Danish Patient Registry for cancers diagnosed from 1976 to May 9, 2009. Information about cancer deaths in the period from 1976 to May 9, 2009, was obtained from the national Danish Civil Registration System and the national Danish Causes of Death Registry. Cancer diagnoses in all registers were classified according to the World Health Organization (WHO) *International Classification of Diseases, Seventh Revision* (*ICD*-7) (22) and *Tenth Revision* (*ICD-10*) (23) codes as follows: any cancer (*ICD*-7 codes 140–205, *ICD-10* codes C00–D09); gastrointestinal cancer, including oral, esophageal, stomach, small intestine, liver, biliary tract, pancreatic, colon, rectal, and anal cancers (*ICD*-7 codes 140–159, *ICD-10* codes C00–C26); hematological cancer, including non-Hodgkin lymphoma, Hodgkin disease, multiple myeloma, and leukemia (*ICD*-7 codes 200–209, *ICD-10* codes C81–C96); respiratory cancer, including laryngeal and lung cancer (*ICD*-7 codes 160–163, *ICD-10* codes C30–C39); urological cancer, including kidney and bladder cancer (*ICD*-7 codes 188–189, *ICD-10* codes C64–C68); female-specific cancers, including breast, cervix uteri, corpus uteri, ovarian, and vaginal cancers (*ICD*-7 codes 174 and 180–184, *ICD-10* codes C50–C58); and male-specific

cancers, including testicular and prostate cancers (*ICD*-7 codes 185–187, *ICD-10* codes C60–C65).

Genotype Analyses

We used a Prism 7900HT Sequence Detection System (Applied Biosystems, Inc, Foster City, CA) to genotype four coding single-nucleotide polymorphisms in three genes: *PCSK9* R46L (rs11591147) (24), *ABCG8* D19H (rs11887534) (25), and *APOE* R112C (rs429358) and R158C (rs7412) (26). All variants are known to reduce LDL cholesterol levels, but their exact effects on protein function are not known. *APOE* R112C and R158C define three major apolipoprotein E isoforms: ApoE-ε2 (cysteine at positions 112 and 158), ApoE-ε3 (cysteine at positions 112 and arginine at 158), and ApoE-ε4 (arginine at positions 112 and 158) (26). All genotyping was done using standard polymerase chain reaction conditions according to the manufacturer's protocol. Forward (F) and reverse (R) primer sequences and VIC- and 6-FAM-labeled probe sequences, all in the 5′ to 3′ direction, were as follows (the variant is underlined in the probe sequences): *PCSK9* R46L (rs11591147), F = GACGAGGACGGCGACTAC, R = CC GTGCTCGGGTGCTT, VIC = TGCTAGCCTTGCGTTC, 6-FAM = CTAGCCTTGCTTTC; *ABCG8* D19H (rs11887534), F = AGAGGGCTGCCGAAAGG, R = CGACTTCCCATTG CTCACTCA, VIC = ACTCCCCAGGATACCT, 6-FAM = CT CCCCAGCATACCT; *APOE* R112C (rs429358), F = GGAAC TGGAGGAACAACTGACC, R = ACCTCGCCGCGGTACTG, VIC = ATGGAGGACGTGTGC, 6-FAM = TGGAGGACG TGCGC; and *APOE* R158C (rs7412), F = TCCGCGATGCC GATGAC, R = CCCCGGCCTGGTACAC, VIC = CAGGCG CTTCTGC, 6-FAM = CAGGCACTTCTGC. Because we performed reruns, 99.9% of all available participants were genotyped for all four polymorphisms. Genotypes for each polymorphism were verified by sequencing 20–30 randomly selected DNA samples.

Biochemical Analyses

We used colorimetric assays (Boehringer Mannheim, Mannheim, Germany or Kone-lab, Espoo, Finland) to measure total cholesterol, high-density lipoprotein cholesterol, and triglycerides in plasma according to the manufactures' protocols. Plasma total cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured on the same day when the blood sample was collected. Plasma levels of LDL cholesterol were calculated by using the Friedewald equation (27) in samples in which the triglyceride level was less than 354 mg/dL (4 mmol/L); for samples with higher triglyceride levels, the plasma level of LDL cholesterol was measured directly on the same day or from samples frozen at −80°C.

Other Covariates

Statistical analyses were adjusted for covariates that could potentially influence LDL cholesterol levels and/or the risk of cancer. Information on covariates was obtained from physical examinations and questionnaires administered at one or more study examinations and included BMI, which was calculated as weight in kilograms divided by height in meters squared. Categorical covariates were recorded as absent or present as follows: hypertension [systolic blood pressure ≥140 mm Hg (≥135 mm Hg for diabetics) and/or diastolic blood pressure ≥90 mmHg (≥85 mmHg for diabetics), according to current WHO criteria (28), and/or use of antihypertensive medication prescribed specifically for hypertension], diabetes mellitus [self-reported diabetes and/or use of antidiabetic medication and/or a nonfasting plasma glucose >11.0 mmol/L, according to current WHO criteria (29), and/or hospitalization or death due to diabetes defined by the WHO *International Classification of Diseases, Eighth Revision* (*ICD-8*) (30) codes 249 and 250 and *ICD-10* codes E10–E11 and E13–E14], current smoking, ischemic heart disease (hospitalization or death due to ischemic heart disease defined by *ICD-8* codes 410–414 and *ICD-10* codes I20–I25), and statin use (self-reported use of lipid-lowering medication).

Statistical Analysis

Data were analyzed using Stata/SE statistical software (version 10.1; StataCorp, College Station, TX). *P* values less than .05 were considered statistically significant, and all statistical tests were two-sided. For trend tests, groups of individuals classified by plasma LDL cholesterol levels or genotypes were ranked according to decreasing known LDL cholesterol levels. Groups were coded by rank as 0, 1, and 2 for *PCSK9* and *ABCA8*, and 0, 1, 2, 3, 4, 5 for *APOE*. Expected levels of LDL cholesterol were known from our previous studies and the literature (24,25,31).

We conducted the following four analyses to study the potential causal association of low LDL cholesterol levels with risk of cancer. First, to test whether low plasma LDL cholesterol levels were associated with an increased risk of cancer, we used Cox proportional hazards regression models, with age as the time scale and use of left truncation (delayed entry), to estimate the hazard ratios of cancer in the CCHS; these analyses were conducted using data for the first plasma LDL cholesterol measurement in the 1991–1994 or 2003–2004 examinations (baseline), and data from subsequent follow-up examinations were used as time-dependent covariates for multifactor adjustment. We tested the assumption of proportional hazards in all Cox regression analyses by plotting –ln(survival probability) against ln(analysis time), and by the use of the Schoenfeld test (32); these tests verified the assumption of proportional hazards. In the CGPS, logistic regression analysis was used to test the association between plasma LDL cholesterol levels and risk of cancer with only one measurement of covariates for multifactor adjustment was available since participants in this study are only examined once. We estimated the risk of cancer as a function of plasma LDL cholesterol levels by categorizing LDL cholesterol levels according to the Adult Treatment Panel III (ATP) categories (plasma LDL cholesterol <100, 100–119.9, 120–129.9, 130–159.9, or ≥160 mg/dL) (33) or as percentile groups (plasma LDL cholesterol <10th, 10th–33rd, 34th– 66th, or >66th percentile) or by treating plasma LDL cholesterol level as a continuous variable; the hazard ratios were corrected for regression dilution bias (34) for the CCHS but not for the CGPS because individuals were only examined once in this study and adjustment for regression dilution bias requires at least two measurements from each individual and obtained several years apart. Exploratory analyses revealed that the increased risk of cancer was most pronounced for levels of plasma LDL cholesterol below the 33rd percentile; thus, percentile groups were divided into tertiles to ensure a large reference group representative of the population (plasma LDL cholesterol >66th percentile,

158 mg/dL) and the lowest tertile was further divided into plasma LDL cholesterol levels below and above the 10th percentile (<87 mg/dL) to study risk in those with very low (<10th percentile) plasma LDL cholesterol levels separately. Hazard ratios were adjusted for 1) age and sex; 2) age, sex, BMI (continuous variable), hypertension, diabetes mellitus, current smoking, and current statin use; 3) age, sex, BMI, hypertension, diabetes mellitus, current smoking, current statin use, and ischemic heart disease; 4) age, sex, BMI, hypertension, diabetes mellitus, current smoking, and current statin use after exclusion of events that occurred within the first 4 years after the plasma LDL cholesterol measurement (CCHS only); and 5) age, sex, BMI, hypertension, diabetes mellitus, current smoking, and current statin use after exclusion of participants who received statin treatment (CGPS only).

Second, to test whether the *PCSK9*, *ABCG8*, or the *APOE* ε genotypes were associated with low plasma LDL cholesterol levels, we used a nonparametric trend test (35) across genotypes after ranking the *PCSK9*, *ABCG8*, and *APOE* genotypes according to decreasing LDL cholesterol levels as described above.

Third, we examined whether genetically reduced levels of LDL cholesterol were associated with an increased risk of cancer by using a Cox proportional hazards regression model for the CCHS data, a logistic regression model for the CGPS data, and, to obtain maximal statistical power, a logistic regression model for data from the two studies combined. These analyses were adjusted for age, sex, ischemic heart disease, and statin use.

Finally, we conducted instrumental variable analysis by two-stage least squares regression to assess the potential causal relationship between genetically reduced LDL cholesterol levels and an increased risk of cancer using genotypes that are known to be associated with plasma LDL cholesterol as indicator variables for low LDL cholesterol in an additive model. Genotypes were included as unranked indicators in the first-stage regression in the instrumental variable analysis to reduce the risk of overestimating the association between genotypes and LDL cholesterol level. The strengths of the instruments (ie, the associations between genotype and plasma LDL cholesterol) were evaluated by the use of *F* statistics from the first-stage regression, with an *F* statistic greater than 10 indicating sufficient strength to ensure the validity of the instrumental variable analysis; R^2 as a percentage was used as a measure of the percent contribution of genotype to the variation in LDL cholesterol (12). The risk of cancer associated with a 50% reduction in LDL cholesterol (either as measured in plasma or due to genetic polymorphisms) was calculated using logarithms of LDL cholesterol to base 2 in regression models, multiplying the regression coefficients by −1, and exponentiating the product to give hazard ratios and odds ratios. We used the Altman and Bland method (36) to compare the causal estimate obtained from the instrumental variable analysis with the fully adjusted (including genotype) observational increased risk of cancer from conventional epidemiology, thus taking into account the covariance between the two estimators.

Results

Characteristics of participants, as a function of the percentile group of plasma LDL cholesterol level, are shown in Table 1. Individuals with low plasma LDL cholesterol levels were younger and had a lower BMI and a lower occurrence of hypertension compared with individuals with high levels.

Plasma LDL Cholesterol Level and Risk of Cancer

Low plasma levels of LDL cholesterol were associated with an increased risk of cancer in the CCHS (Figure 1) and CGPS (Figure 2), both as a function of ATP categories and as a function of percentile groups. In the CCHS, compared with plasma LDL cholesterol levels greater than the 66th percentile (>158 mg/dL), those lower than the 10th percentile (<87 mg/dL) were associated with a 43% increase (95% confidence interval [CI] = 15% to 79% increase) in the risk of cancer. We obtained similar corresponding risk increases after multifactor adjustment (41% increase, 95% CI = 14% to 76% increase), after further adjustment for ischemic heart disease (40% increase, 95% CI = 13% to 74% increase), and after exclusion of events that occurred within 4 years after blood sampling (43% increase, 95% CI = 11% to 84% increase) (Figure 1). In the CGPS, compared with plasma LDL cholesterol levels greater than the 66th percentile (>135 mg/dL), those lower than the 10th percentile (<80 mg/dL) were associated with a 31% increase (95% CI = 18% to 45% increase) in the risk of cancer, and, after exclusion of statin-treated participants in the CGPS, with a 58% increase (95% CI = 39% to 81% increase) (Figure 2). In the CCHS, 50% reduction in plasma LDL cholesterol level was associated with a 10% increase (95% CI = 1% to 21% increase; $P = .003$) in risk of cancer in a multifactor-adjusted analysis (Table 2).

We next examined the risks of specific types of cancer by percentile group of plasma LDL cholesterol level. In the CCHS, plasma LDL cholesterol levels below the 10th percentile (<87 mg/dL) vs those above the 66th percentile (>158 mg/dL) were associated with a 61% increase (95% CI = 9% to 136% increase) in the risk of gastrointestinal cancer and with slightly smaller, albeit non-statistically significant effect sizes for the risks of hematological cancer (42% increase, 95% CI = 45% decrease to 367% increase), respiratory cancer (41% increase, 95% CI = 12% decrease to 225% increase), female-specific cancers (41% increase, 95% CI = 10% decrease to 219% increase), and male-specific cancers (34% increase, 95% CI = 37% decrease to 285% increase) (Table 3). In the CGPS, plasma LDL cholesterol levels below the 10th percentile (<80 mg/dL) vs those above the 66th percentile (>135 mg/dL) were associated with 83% increase (95% CI = 43% to 133% increase) in the risk of gastrointestinal cancer, a 95% increase (95% CI = 30% to 192% increase) in the risk of hematological cancer, a 108% increase (95% CI = 34% to 224% increase) in the risk of urological cancer, and a 26% increase (95% CI = 5% to 51% increase) in the risk of female-specific cancers, with slightly smaller non-statistically significant effect sizes for respiratory cancer and for other cancers (Table 3).

Genotypes and Plasma LDL Cholesterol Levels

To study the effect sizes of genotypes on plasma LDL cholesterol levels and ensure that the genotypes were valid as indicators (or instruments) for lifelong low LDL cholesterol, we plotted mean plasma LDL cholesterol levels and 95% confidence intervals as a function of genotype. Compared with the corresponding

Table 1. Characteristics of participants by percentile groups of low-density lipoprotein (LDL) cholesterol*

Characteristic	LDL cholesterol percentile group†				P_{trend}‡
	<10th	10th–33rd	34th–66th	>66th	
Copenhagen City Heart Study					
No. of participants	1061	2473	3525	3554	
Median plasma LDL cholesterol (IQR), mg/dL	74 (65–81)	107 (98–114)	139 (129–148)	185 (170–206)	<.001
Median age (IQR), y	39 (29–56)	49 (35–65)	58 (46–69)	64 (55–72)	<.001
Women, %	59	55	52	59	<.001
Median BMI (IQR), kg/m^2	23 (21–25)	24 (22–27)	25 (23–28)	26 (23–29)	<.001
Hypertension, %	36	49	62	70	<.001
Diabetes mellitus, %	2.6	3.7	3.5	3.4	.44
Current smoker, %	44	44	48	48	.001
Ischemic heart disease, %	10	14	19	27	<.001
Statin user, %	1.2	0.8	1.1	1.1	.49
Copenhagen General Population Study					
No. of participants	5862	13 052	19 652	21 000	
Median plasma LDL cholesterol (IQR), mg/dL	70 (58–73)	97 (89–101)	120 (112–128)	159 (147–178)	<.001
Median age (IQR), y	58 (44–70)	55 (44–66)	58 (47–66)	59 (50–67)	<.001
Women, %	46	42	45	46	<.001
Median BMI (IQR), kg/m^2	25 (22–28)	25 (23–28)	26 (23–28)	26 (24–29)	<.001
Hypertension, %	67	63	68	75	<.001
Diabetes mellitus, %	13.8	4.9	2.6	1.6	<.001
Current smoker, %	18	19	21	24	<.001
Ischemic heart disease, %	17	9	5	5	<.001
Statin user, %	34.1	15.6	6.9	2.6	<.001

* Data are from the 1991–1994 or 2001–2003 examinations of the Copenhagen City Heart Study when DNA was collected and LDL cholesterol measured (=baseline) and from study enrollment in 2003–2009 for the Copenhagen General Population Study. BMI = body mass index; IQR = interquartile range .

† Tertile groups of plasma LDL cholesterol levels were used to obtain a sufficiently large reference group representative for the population. The lowest tertile was divided into plasma LDL cholesterol levels below and above the 10th percentile to be able to study the effects of very low levels.

‡ Nonparametric test by Cuzick (35) for continuous variables and by Cuzick extension (35) of a Wilcoxon rank-sum test for categorical variables (all tests two-sided).

noncarriers, *PCSK9* R46L homozygotes had a 20% reduction (95% CI = 1% to 39% reduction) in plasma LDL cholesterol levels (P_{trend} across genotypes < .001) and *ABCG8* D19H homozygotes had a 5% reduction (95% CI = 1% to 8% reduction) (P_{trend} across genotypes < .001). Compared with *APOE* ε44 genotype, the *APOE* ε22 genotype was associated with a 38% reduction (95% CI = 36% to 41% reduction) in plasma LDL cholesterol in the CCHS and CGPS combined (P_{trend} across genotypes < .001) (Figure 3). Sixty percent of *PCSK9* R46L homozygotes, 24% of *ABCG8* D19H homozygotes, and 66% of *APOE* ε22 genotype carriers had a plasma LDL cholesterol level below 100 mg/dL, corresponding to the lowest ATP group. *PCSK9* R46L, *ABCG8* D19H, and *APOE* ε genotypes contributed 0.4%, 0.1%, and 5.9%, respectively, to the total variation in plasma LDL cholesterol (P < .001; Table 2).

Genotypes and the Risk of Cancer

Assuming that low plasma LDL cholesterol levels is causally associated with an increased risk of cancer, we reasoned that genetically reduced LDL cholesterol levels should confer a similar increase in the risk of cancer as that observed for low plasma LDL cholesterol levels in the CCHS and CGPS. For example, the 38% reduction in plasma LDL cholesterol level observed for the *APOE* ε22 genotype vs the *APOE* ε44 genotype would theoretically predict 14% increased risk of cancer (odds ratio = 1.14, 95% CI = 1.03 to 1.26) (Figure 3, middle panel). However, the observed risk of cancer as a function of genotype in the CCHS and CGPS combined did not differ statistically significantly from 1.0 for the *ABCG8* D19H genotype (P_{trend} = .78) or the *APOE* genotype (P_{trend} = .96) (Figure 3, right panel). (Because there were only three cancer events among *PCSK9* R46L homozygotes, risk was not estimated and a trend test not performed.) In addition, when we used the most common genotype—*APOE* ε33—as the reference group instead of *APOE* ε44, none of the *APOE* genotypes was associated with the risk of cancer (data not shown). These findings were confirmed for all subgroups of cancer (P_{trend} ranged from .07 to .96; Supplementary Table 1, available online). Moreover, the various risk factors for low plasma LDL cholesterol and for cancer were equally distributed among the different genotypes (Supplementary Table 2, available online), confirming that the genotypes are not confounded. Excluding statin users from analyses of association between genotypes and cancer risk did not substantially change the risk estimates (data not shown).

Potential Causal Effect of Low LDL Cholesterol on the Risk of Cancer

We also examined the potential causal association between low LDL cholesterol levels and an increased risk of cancer by using instrumental variable analysis and generalized least squares regression. The causal odds ratio for cancer for a 50% reduction in plasma LDL cholesterol level due to all the genotypes in both studies combined was 0.96 (95% CI = 0.87 to 1.05), whereas the hazard ratio of cancer for a 50% reduction in plasma LDL cholesterol level in the

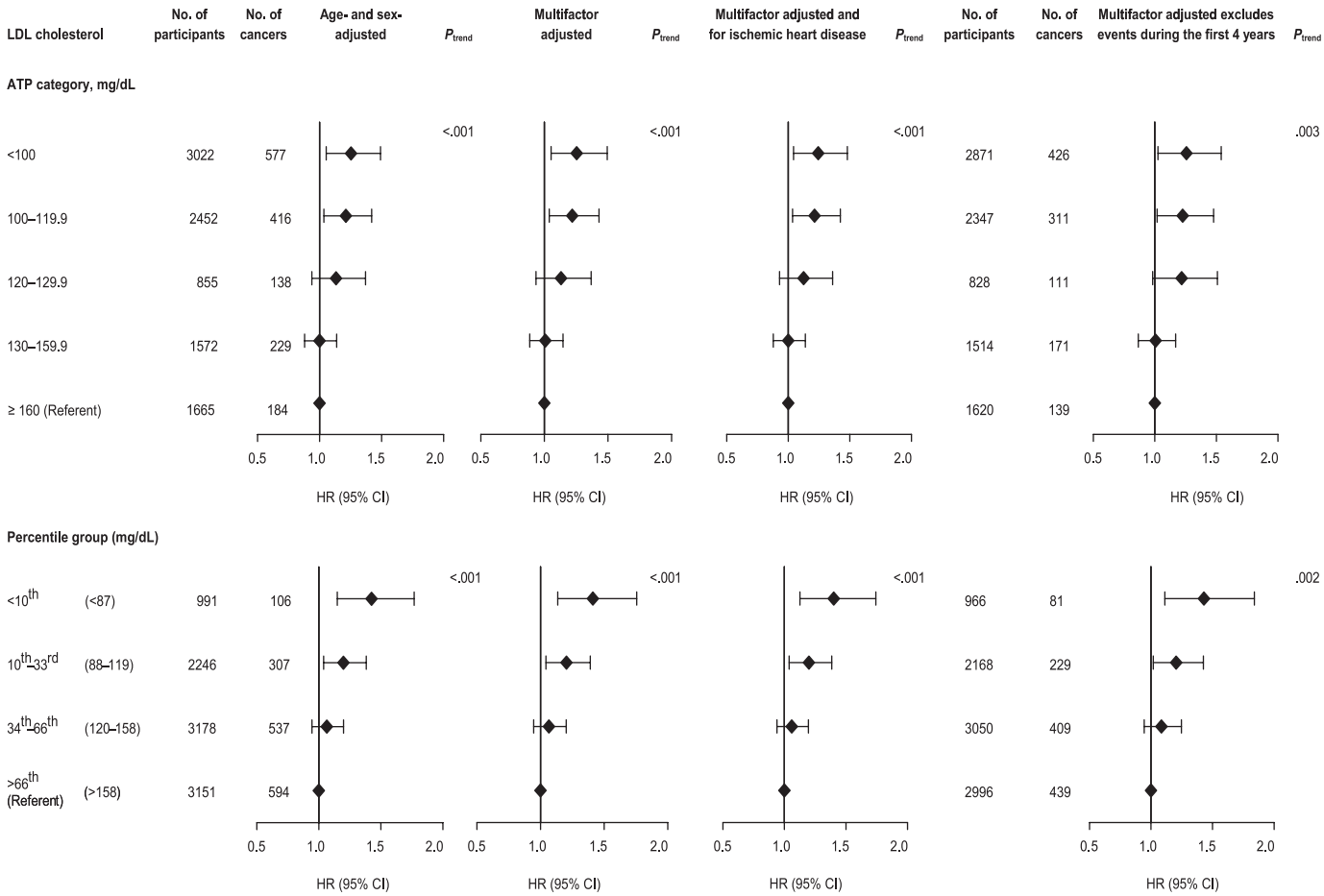

Figure 1. Risk of any cancer as a function of plasma low-density lipoprotein (LDL) cholesterol level in the Copenhagen City Heart Study. Plasma LDL cholesterol levels were measured in 11 110 individuals who participated in the 1991–1994 or the 2001–2003 examinations of the Copenhagen City Heart Study and were subsequently followed up for a median of 15 years with respect to incident cancer. Individuals with cancer before study entry were excluded, explaining why the number of participants is lower than the overall number of participants with a LDL measurement. Multifactor adjustment was for age, sex, body mass index, hypertension, diabetes mellitus, current smoking, and statin use. **Black diamonds** represent the hazard ratios, and **error bars** indicate the 95% confidence intervals (CIs). *P* values for trend are two-sided and were estimated by Cuzick extension of a Wilcoxon rank-sum test. ATP = adult treatment panel III .

CCHS was 1.10 (95% CI = 1.01 to 1.21) (*P* for causal odds ratio vs observed hazard ratio = .03) (Figure 4). The corresponding causal odds ratio of cancer for a 50% reduction in plasma LDL cholesterol level due to *APOE* genotype with largest effect on plasma LDL cholesterol as a single genotype was 0.92 (95% CI = 0.75 to 1.10) (*P* for causal odds ratio vs observed hazard ratio = .02). Table 2 shows the corresponding causal odds ratio for each genotype as well as the *F* statistic, a measure of strength of each genotype as an instrumental variable (an *F* statistic >10 indicates an instrument of sufficient strength). *F* statistics was 131 for *PCSK9* R46L genotype, 23 for *ABCG8* D19H genotype, 844 for *APOE* genotypes, and 474 for all genotypes combined (Table 2).

Discussion

The main finding of this study was that low plasma levels of LDL cholesterol were robustly associated with an increased risk of cancer, but that genetically reduced LDL cholesterol levels (due to polymorphisms that are associated with lifelong reduced plasma LDL cholesterol levels) were not. This finding suggests that low LDL cholesterol levels per se do not cause cancer.

Whether low plasma LDL cholesterol increases the risk of cancer is an important issue; even more important is whether an intervention that decreases plasma LDL cholesterol level increases the risk of cancer. One motivation for this study was the recently reported findings of the SEAS trial, which suggested that a reduction of plasma LDL cholesterol is associated with an increased risk of cancer (37). However, a meta-analysis of data from three trials of simvastatin and ezetimibe (including the SEAS trial) found no evidence that decreasing plasma levels of LDL cholesterol increases the risk of cancer (8).

Several epidemiological studies have reported that low plasma LDL cholesterol levels are associated with an increased risk of cancer (1–6). In this study, we showed that this association is robust: the risk estimates were similar after adjustment for multiple risk factors, including ischemic heart disease, and after exclusion of events that occurred within 4 years after blood sampling or participants who were treated with statins. Our observational data of the association between plasma LDL cholesterol levels and risk of cancer (Figures 1 and 2) show that it is difficult to eliminate the association between low plasma LDL cholesterol level and the risk of cancer by statistical adjustment alone. Nonetheless, three polymorphic genotypes

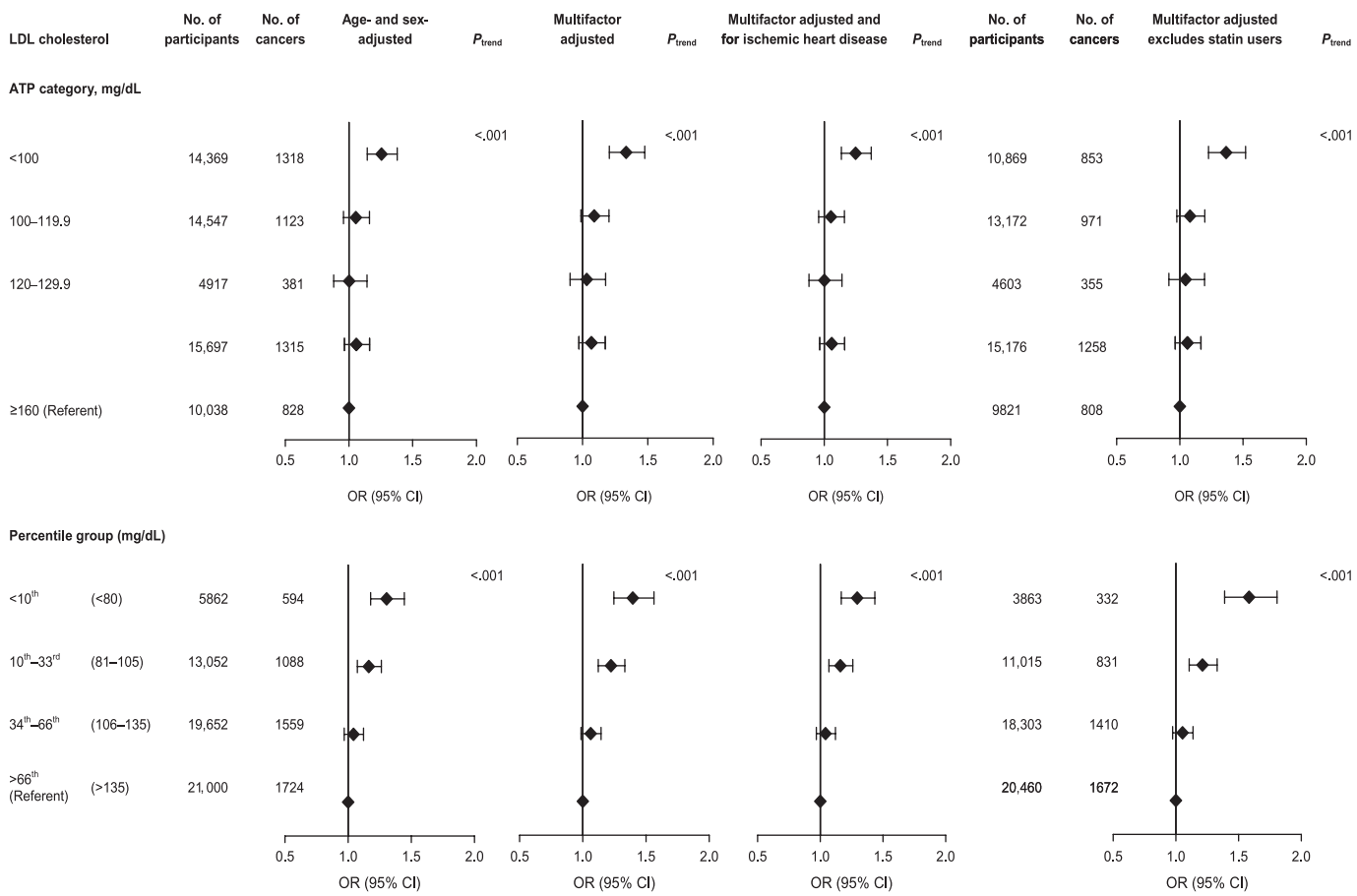

Figure 2. Risk of any cancer as a function of plasma low-density lipoprotein (LDL) cholesterol level in the Copenhagen General Population Study. Plasma LDL cholesterol levels were measured in 59 566 individuals at inclusion into the study in 2003–2009. Multifactor adjustment was for age, sex, body mass index, hypertension, diabetes mellitus, current smoking, and statin use (except in the analysis that excluded statin users). **Black diamonds** represent the odds ratio (HRs), and **error bars** indicate the 95% confidence intervals (CIs). *P* values for trend are two-sided and were estimated by Cuzick extension of a Wilcoxon rank-sum test. ATP = adult treatment panel III.

in *PCSK9*, *ABCG8*, and *APOE*, each of which is associated with lifelong decreased plasma LDL cholesterol levels, were not associated with an increased risk of cancer. The difference between the observational and genetic data suggests that one often cannot accomplish complete statistical control in observational studies.

By using a Mendelian randomization approach, which circumvents reverse causation and confounding (12–17), we also showed that a 50% reduction in LDL cholesterol caused by genotypes was not associated with an increased risk of cancer, either overall or for any cancer subtype, even though a 50% reduction in plasma LDL

Table 2. Studies summary of the causal effect of reduced low-density lipoprotein (LDL) cholesterol on increased risk of cancer*

Model	*F*	R^2, %	Relative risk† (95% CI) of cancer for a 50% reduction in LDL cholesterol level	*P*‡	*P*§
Observational estimate	—	—	1.10 (1.01 to 1.21)	.003	—
Instrumental variable estimate					
All genotypes combined	474	6.5	0.96 (0.87 to 1.05)	.31	.03
PCSK9 R46L	131	0.4	1.33 (0.59 to 2.97)	.30	.42
ABCG8 D19H	23	0.1	2.28 (0.43 to 12.11)	.73	.23
APOE genotype	844	5.9	0.92 (0.75 to 1.10)	.39	.02

* *F* statistics (evaluation of strength of instrument) and R^2 (contribution of genotype to variation in LDL cholesterol levels in percent) are from the first-stage regression analysis. — = not applicable; CI = confidence interval.

† The observational estimate of risk is a hazard ratio; the instrumental variable estimates of risk are odds ratios.

‡ For the statistical significance of the hazard ratio or odds ratio from Cox proportional hazards or logistic regression analysis (two-sided).

§ For the observational estimate from conventional epidemiology vs the causal estimate from instrumental variable analysis by the method Altman and Bland (36) (two-sided).

Table 3. Risk of specific types of cancer by percentile group of low-density lipoprotein (LDL) cholesterol*

Cancer type	LDL cholesterol percentile group				P_{trend}†
	<10th	10th–33rd	34th–66th	>66th	
Copenhagen City Heart Study					
No. of participants	991	2246	3178	3151	
Plasma LDL cholesterol, mg/dL	<87	88–119	120–158	>158	
Gastrointestinal cancer, HR (95% CI)	1.61 (1.09 to 2.36)	1.27 (0.98 to 1.65)	1.10 (0.89 to 1.37)	1.00 (referent)	.009
No. of cancers	33	96	160	176	
Hematological cancer, HR (95% CI)	1.42 (0.55 to 3.67)	1.66 (0.96 to 2.88)	0.99 (0.59 to 1.65)	1.00 (referent)	.11
No. of cancers	6	22	30	33	
Respiratory cancer, HR (95% CI)	1.41 (0.88 to 2.25)	1.21 (0.88 to 1.65)	0.91 (0.69 to 1.20)	1.00 (referent)	.12
No. of cancers	21	67	96	123	
Urological cancer, HR (95% CI)	0.66 (0.24 to 1.84)	0.67 (0.37 to 1.22)	0.81 (0.52 to 1.26)	1.00 (referent)	.14
No. of cancers	6	16	34	47	
Female-specific cancer, HR (95% CI)	1.41 (0.90 to 2.19)	1.03 (0.76 to 1.41)	0.86 (0.66 to 1.12)	1.00 (referent)	.38
No. of cancers	27	65	100	149	
Male-specific cancer, HR (95% CI)	1.34 (0.63 to 2.85)	1.06 (0.65 to 1.75)	1.16 (0.78 to 1.73)	1.00 (referent)	0.52
No. of cancers	8	24	56	46	
Other cancers, HR (95% CI)	1.14 (0.61 to 2.14)	1.53 (1.05 to 2.22)	1.51 (1.10 to 2.06)	1.00 (referent)	.08
No. of cancers	16	51	98	78	
Any cancer, HR (95% CI)	1.41 (1.14 to 1.76)	1.20 (1.04 to 1.39)	1.07 (0.95 to 1.20)	1.00 (referent)	<.001
No. of cancers	106	307	537	594	
Copenhagen General Population Study					
No. of participants	5862	13052	19652	21000	
Plasma LDL cholesterol, mg/dL	<80	81–105	106–135	>135	
Gastrointestinal cancer, OR (95% CI)	1.83 (1.43 to 2.33)	1.50 (1.24 to 1.82)	1.14 (0.96 to 1.36)	1.00 (referent)	<.001
No. of cancers	130	214	272	273	
Hematological cancer, OR (95% CI)	1.95 (1.30 to 2.92)	1.53 (1.11 to 2.10)	1.10 (0.83 to 1.48)	1.00 (referent)	<.001
No. of cancers	40	74	89	99	
Respiratory cancer, OR (95% CI)	1.31 (0.88 to 1.94)	1.23 (0.91 to 1.68)	0.95 (0.72 to 1.26)	1.00 (referent)	.10
No. of cancers	48	82	93	110	
Urological cancer, OR (95% CI)	2.08 (1.34 to 3.24)	1.98 (1.40 to 2.81)	1.34 (0.96 to 1.86)	1.00 (referent)	<.001
No. of cancers	42	75	83	67	
Female-specific cancer, OR (95% CI)	1.26 (1.05 to 1.51)	1.09 (0.95 to 1.25)	1.02 (0.90 to 1.14)	1.00 (referent)	.02
No. of cancers (women only)	204	405	604	722	
Male-specific cancer, OR (95% CI)	1.09 (0.82 to 1.44)	0.93 (0.74 to 1.16)	0.85 (0.70 to 1.03)	1.00 (referent)	.92
No. of cancers (men only)	96	146	213	247	
Other cancers, OR (95% CI)	1.24 (0.97 to 1.58)	1.04 (0.86 to 1.25)	1.11 (0.95 to 1.29)	1.00 (referent)	.17
No. of cancers	110	196	330	344	
Any cancer, OR (95% CI)	1.40 (1.25 to 1.56)	1.22 (1.12 to 1.33)	1.06 (0.99 to 1.15)	1.00 (referent)	<.001
No. of cancers	594	1088	1559	1724	

* The hazard ratios in the Copenhagen City Heart Study and the odds ratios in the Copenhagen General Population Study were adjusted for age, sex, body mass index, hypertension, diabetes mellitus, smoking, and statin use. Number of cancers in the different groups added together is larger than the number of any cancer because some participants developed more than one type of cancer. CI = confidence interval; HR = hazard ration; OR = odds ratio.

† Two-sided P_{trend} by Cuzick extension of a Wilcoxon rank-sum test (35).

cholesterol level was associated with a 10% increased risk of cancer in observational epidemiology. This finding indicates that low LDL cholesterol levels are secondary to a preclinical cancer or that a confounding factor causes both low plasma LDL cholesterol and an increased risk of cancer. This conclusion is in accordance with results from the Atherosclerosis Risk in Communities cohort study (n = 13 250) (38) and a study of elderly individuals treated with pravastatin (n = 2913) (39).

How preclinical cancers might cause low plasma LDL cholesterol levels is not known. Potential mechanisms include effects on cholesterol absorption, transport, metabolism, or utilization. In support of the notion that preclinical cancers increase LDL cholesterol metabolism or utilization, previous studies have shown that plasma cholesterol levels are inversely associated with tumor mass of hematological cancers and that plasma cholesterol levels revert to normal after cancer remission (40,41). This idea is also supported by the specific cancer subtypes that have been observed to be associated with low LDL cholesterol levels in this and previous studies, that is, gastrointestinal cancer, hematological cancer, female-specific cancers, urological cancer (1,2,4), and lung cancer (2,4). All of these cancers have the potential to produce large tumors and metastases and can remain preclinical for a considerable time before diagnosis. It is also important to consider confounding by a factor that causes both a low plasma LDL cholesterol level and increases the risk of cancer. One potential confounder is severe alcoholism, which leads to both nutritionally caused low plasma LDL cholesterol levels and an increased risk of liver cancer along with liver cirrhosis. Another potential confounder is smoking, which could lead to both an increased risk of cancer and low plasma LDL cholesterol levels via, for example, chronic obstructive

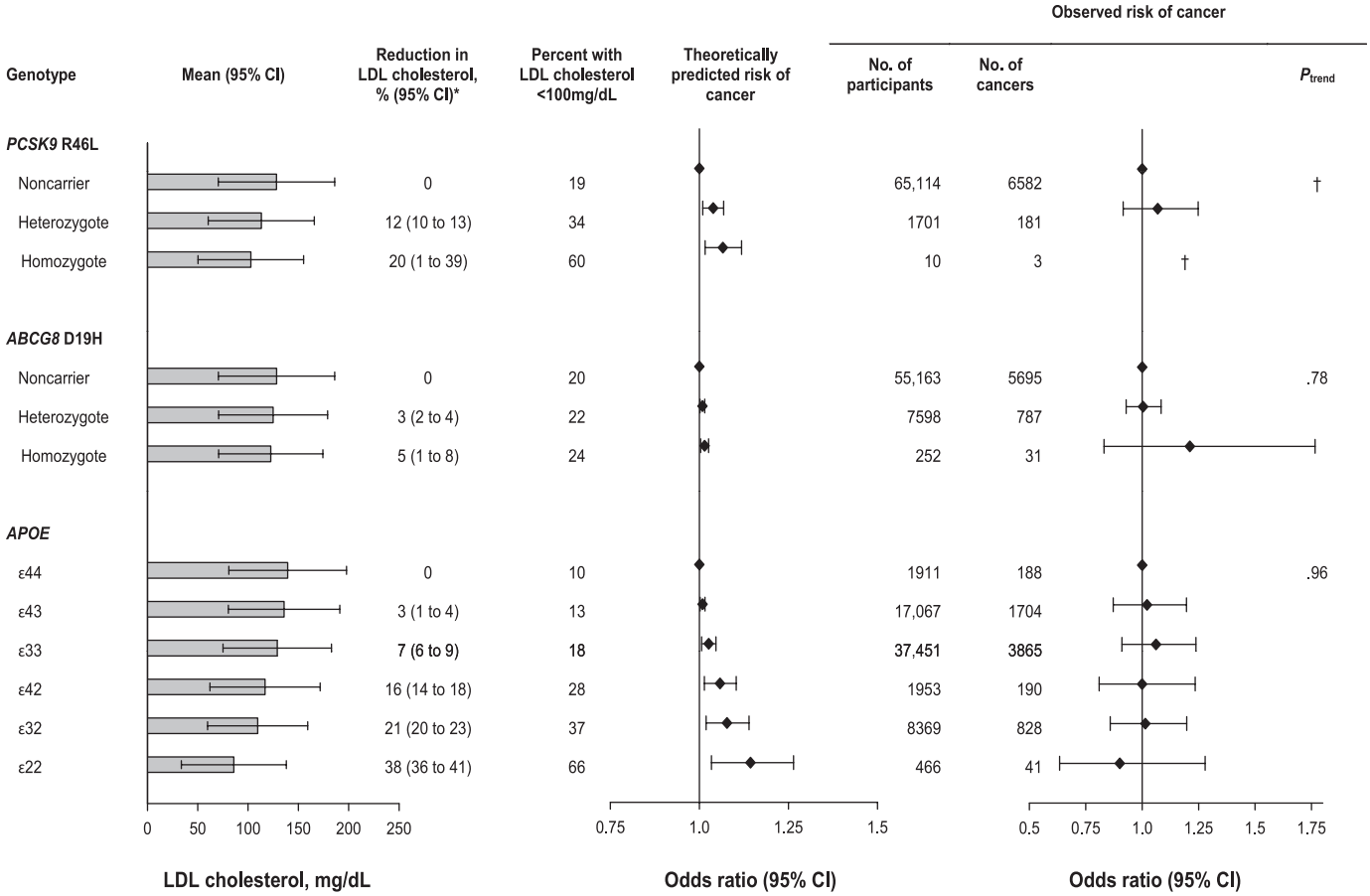

Figure 3. Low-density lipoprotein (LDL) cholesterol level and the risk of cancer as a function of *PCSK9, ABCG8,* and *APOE* genotypes. This association was tested in 67 507 individuals from the general population who participated in the Copenhagen City Heart Study and the Copenhagen General Population Study using the genotype associated with the highest plasma LDL cholesterol levels as the referent group. Odds ratios were adjusted for age, sex, ischemic heart disease, and statin use. *Reduction in plasma LDL cholesterol in percent is relative to the genotype with the highest LDL cholesterol level. †Odds ratio for *PCSK9* homozygosity (n = 10) and was not calculated due to the low number of cancer cases among homozygotes (n = 3) and thus a high uncertainty of risk estimate. P_{trend} across *PCSK9* genotype was not calculated because only estimates for noncarriers and heterozygotes were included, only resulting in two groups. *P* values for trend were estimated by Cuzick extension of a Wilcoxon rank-sum test (35). **Black diamonds** represent the odds ratios, and **error bars** indicate the 95% confidence intervals (CIs).

pulmonary disease followed by poor nutritional status and an increased risk of lung cancer.

Using a Mendelian randomization approach to study a cause-and-effect relationship that is suggested by observational epidemiology such as the one between low plasma LDL cholesterol levels and an increased risk of cancer reduces the risk of regression dilution bias, confounding, and reverse causation; however, three conditions must be fulfilled (12–15,17). First, the genotypes (instrumental variable) must be associated with the exposure variable, that is, with low plasma LDL cholesterol levels. Although several genetic variants are associated with elevated plasma LDL cholesterol levels, including mutations in the LDL receptor gene and in its ligand apolipoprotein B (42–44), only a few variants are associated with low plasma LDL cholesterol levels. For this study, we selected genetic variants that are specifically associated with low plasma LDL cholesterol levels (24,25,31). This is important because using variants associated with elevated levels of LDL cholesterol rather than with reduced levels may introduce bias regarding the selection of the referent group because variants associated with high plasma LDL cholesterol levels may also be associated with other phenotypic traits and diseases (pleiotropic effects). The *APOE* ε genotype used in this study contributed 5.9% to the total variation in plasma LDL cholesterol, which is almost half of the total 12.4% variation in LDL cholesterol explained by all genetic variants known so far (42) and the genotype was, according to the *F* statistic, a strong instrument (*F* statistic = 844), *APOE* ε22 was associated with up to a 38% reduction in plasma LDL cholesterol levels and 66% of individuals with the ε22 genotype had a plasma LDL cholesterol lower than 100 mg/dL. One may argue that the association in the instrumental variable analysis was mainly driven by the *APOE* genotype; however, from the theoretically predicted risk of cancer calculated for each genotype using the mean plasma LDL cholesterol level, both *PCSK9* heterozygosity and homozygosity, *ABCG8* homozygosity, and *APOE* ε33, ε42, ε32, and ε22 genotypes should confer increased risk of cancer if low LDL cholesterol levels were causal.

Second, the genotypes must be independent of factors that confound the association between plasma LDL cholesterol levels

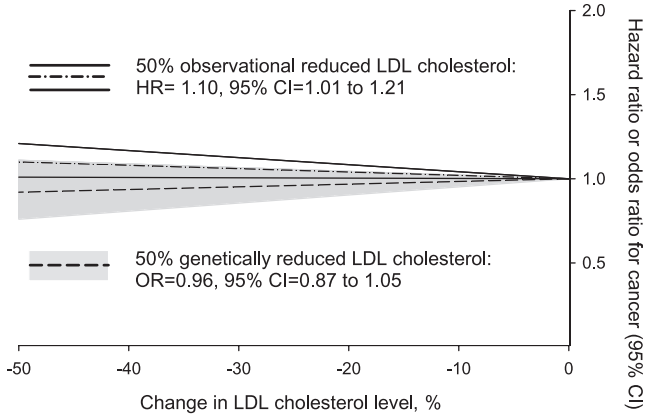

Figure 4. Summary of the causal effect of reduced low-density lipoprotein (LDL) cholesterol on the risk of cancer. The causal effect was tested in 67 507 individuals from the general population who participated in the Copenhagen City Heart Study and the Copenhagen General Population Study. The causal effect of a reduced LDL cholesterol level on the risk of cancer was estimated by the association between genetically reduced LDL cholesterol levels and the risk of cancer, using instrumental variable analysis by two-stage least squares regression and is presented as the odds ratio (**black broken line)** with 95% confidence intervals (**gray shaded area**). This risk is compared with the observed increased cancer risk associated with reduced LDL cholesterol in the Copenhagen City Heart Study and is presented as the hazard ratio (**black slash-dot line**) with 95% confidence intervals (CIs) (**solid lines**). HR = hazard ratio; OR = odds ratio.

and the risk of cancer. This condition was fulfilled in the CCHS and in the CGPS for known cancer risk factors as well as other characteristics (Supplementary Table 2, available online); however, we naturally cannot exclude confounding from unknown and/or unmeasured factors, although such confounding is generally not thought to exist in a Mendelian randomization study (12–14).

Third, the genotypes must be independent of the outcome, that is, they must not affect the risk of cancer by pathways other than through the plasma LDL cholesterol level (12,14). The potential magnitude of the latter problem is difficult to assess because many genes are pleiotropic (ie, they affect multiple phenotypic traits) or the effects of variants may be under some developmental compensation (ie, damage to one gene is compensated by other genes during development). To our knowledge, the *PCSK9* and *APOE* genes have never been associated with risk of cancer. However, *APOE* is associated with numerous other diseases (45) and may have other pleiotropic effects that could indirectly affect the risk of cancer. For example, the *APOE* genotypes could change the diagnostic pattern (ie, earlier diagnosis or under diagnosis) of cancer in individuals with Alzheimer disease, which is more common among carriers of the ϵ4 allele (46). Homozygosity for the *ABCG8* D19H variant is associated with an increased risk of biliary tract cancer (25), probably due to an increased concentration of cholesterol in the gall bladder; however, the magnitude of this problem in this study is likely to be limited because of the low frequency of *ABCG8* D19H homozygotes (ie, 0.4% = 252/62 786; Figure 3) in the study population.

The main limitation of this study is that the genes we used as instrumental variables explain only approximately half (6.5% of 12.4%) of the total genetic variation in plasma LDL cholesterol level. Although we showed that each of the three genotypes included in the Mendelian randomization analyses was a strong instrument based on the *F* statistic, this strength was due, in part, to the very large sample sizes. The actual magnitude of the associations between genotypes and plasma LDL cholesterol levels were somewhat modest, at least for *ABCG8* and *PCSK9*, which contributed only 0.1% and 0.4%, respectively, to the variation in plasma LDL cholesterol levels, whereas *APOE* genotype contributed 5.9%. This relatively modest genetically related change in LDL cholesterol levels, combined with the observation of a non-statistically significantly reduced risk of cancer associated with the genotypes, predicted that a genetically induced 50% reduction in LDL cholesterol level should produce a 4% reduction in cancer risk (causal odds ratio for cancer for a 50% reduction in LDL cholesterol = 0.96), whereas an observed 50% reduction in plasma LDL cholesterol was associated with a 10% increased risk of cancer (hazard ratio of cancer for a 50% reduction in LDL cholesterol = 1.10). Additional studies that include genetic variants that contribute to an even larger fraction of the variation in LDL cholesterol levels and that have even more statistical power than this study are needed to yield even more stable Mendelian randomization estimates of this potential causal association.

The best evidence for absence of a causal association between low plasma LDL cholesterol levels and an increased risk of cancer in this study comes from the formal statistical comparison between the Mendelian randomization estimate and the observational estimate, which yielded a *P* of .02 for *APOE* and a *P* of .03 for the three genotypes combined. It is evident from the *F* statistics that *PCSK9* and *ABCG8* genotypes were weak instrumental variables and contributed very little to this analysis; nevertheless, the strong positive association between the *APOE* genotype and plasma LDL cholesterol levels coupled with the lack of an association between the *APOE* genotype and the risk of cancer should indeed call into question the causality of the observational association between low plasma LDL cholesterol and an increased risk of cancer. However, by itself, a causal connection cannot be completely ruled out.

Other potential limitations of this study include selection bias and misclassification of LDL cholesterol levels, genotypes, and cancer diagnoses. The study populations were selected using the national Danish Civil Registration system to draw two random samples from the Danish adult general population without knowledge of individuals' LDL cholesterol levels, genotypes, or cancer diagnoses, which should largely exclude any important selection bias. In addition, follow-up was 100% complete. Some misclassification of plasma markers such as LDL cholesterol is known to occur due to regression dilution bias; however, we were able to correct for this bias in the CCHS because plasma LDL cholesterol levels were measured twice, 10 years apart, in approximately 6300 individuals. Some participants, in particular in the CGPS, had a low plasma LDL cholesterol level because they were taking a statin to lower cholesterol and were thus classified into the low LDL cholesterol group along with participants who had genuine low LDL cholesterol perhaps due to a preclinical cancer, which would tend to make the estimate of the association between plasma LDL cholesterol groups and risk of cancer more conservative; however, even after we excluded of all statin-treated participants in the CGPS from the analysis, low plasma LDL cholesterol was still

associated with an increased risk of cancer. Misclassification of genotype is highly unlikely in this study because all genotypes were verified by sequencing and the call rates were greater than 99.9% due to repeated reruns. Ascertainment and classification of cancer is a potential limitation of this study; however, we identified cancers by using the well-validated Danish Cancer Registry, which captures 98% of all cancers in Denmark (20,21). The final limitation of this study was that all participants were white; therefore, our results may not necessarily apply to other races or ethnicities.

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