

Impact of LDL Cholesterol on Microvascular Versus Macrovascular Disease

A Mendelian Randomization Study



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ABSTRACT

BACKGROUND Low-density lipoprotein cholesterol (LDL-C) is causally associated with a high risk of coronary artery disease. Whether this also holds for a spectrum of peripheral vascular diseases is unknown.

OBJECTIVES The purpose of this study was to determine whether high LDL-C causally relates to risk of retinopathy, neuropathy, chronic kidney disease (CKD), and peripheral arterial disease (PAD) in the general population.

METHODS One-sample Mendelian randomization (MR) of 116,419 Danish individuals, 2-sample MR on summary-level data from the Global Lipid Genetics Consortium (GLGC) (n = 94,595) and the UK Biobank (n = 408,455), and meta-analysis of randomized statin trials (n = 64,134) were performed.

RESULTS Observationally, high LDL-C did not associate with high risk of retinopathy or neuropathy. There were stepwise increases in risk of CKD and PAD with higher LDL-C (both p for trend <0.001), with hazard ratios of 1.05 (95% confidence interval [CI]: 0.97 to 1.13) for CKD, and 1.41 (95% CI: 1.23 to 1.62) for PAD in individuals with LDL-C above the 95th percentile versus below the 50th percentile. In genetic, causal analyses in the Copenhagen studies, the risk ratio of disease for a 1 mmol/l higher LDL-C was 1.06 (95% CI: 0.24 to 4.58) for retinopathy, 1.05 (95% CI: 0.64 to 1.72) for neuropathy, 3.83 (95% CI: 2.00 to 7.34) for CKD, and 2.09 (95% CI: 1.30 to 2.38) for PAD. Summary-level data from the GLGC and the UK Biobank for retinopathy, neuropathy, and PAD gave similar results. For CKD, a 1-mmol/l lower LDL-C conferred a higher eGFR of 1.95 ml/min/1.73 m² (95% CI: 1.88 to 2.02 ml/min/1.73 m²) observationally, 5.92 ml/min/1.73 m² (95% CI: 4.97 to 6.86 ml/min/1.73 m²) genetically, and 2.69 ml/min/1.73 m² (95% CI: 1.48 to 3.94 ml/min/1.73 m²) through statin therapy.

CONCLUSIONS High LDL-C was not causally associated with risk of retinopathy and neuropathy; however, high LDL-C was observationally and genetically associated with high risks of PAD and CKD, suggesting that LDL-C is causally involved in the pathogenesis of these diseases. (J Am Coll Cardiol 2019;74:1465–76) © 2019 by the American College of Cardiology Foundation.



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**ABBREVIATIONS
AND ACRONYMS****CKD** = chronic kidney disease**eGFR** = estimated glomerular filtration rate**GLGC** = Global Lipid Genetics Consortium**LDL-C** = low-density lipoprotein cholesterol**MI** = myocardial infarction**MR** = Mendelian randomization**PAD** = peripheral arterial disease

Low-density lipoprotein cholesterol (LDL-C) is causally involved in the pathogenesis of atherosclerosis and high LDL-C levels are causally related to coronary artery disease (1). This causal relationship relies on an extensive body of evidence including genetic, observational, and clinical intervention studies. Whether high LDL-C levels are causally associated with high risk of a spectrum of peripheral microvascular and macrovascular diseases, such as retinopathy, peripheral neuropathy, chronic kidney disease (CKD), and lower extremity peripheral arterial disease (PAD), is less clear. Cardiovascular disease and peripheral vascular diseases often coexist and share several risk factors, such as obesity, type 2 diabetes, hypertension, smoking, and hyperlipidemia. Thus, observational studies examining associations between high LDL-C levels and peripheral vascular diseases are likely to be confounded, and clinical intervention studies evaluating the effect of lipid-lowering drugs on risk of these diseases require a multifactorial intervention approach for ethical reasons, which makes it difficult to estimate the causal contribution of each risk factor. Furthermore, clinical intervention studies are often primarily designed to evaluate cardiovascular endpoints in highly selected groups of patients.

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We tested the hypothesis that high LDL-C levels are causally associated with high risk of retinopathy, peripheral neuropathy, CKD, and PAD in the general population. To examine this, we used Mendelian randomization (MR), an epidemiological approach that utilizes the random assortment of alleles at conception to circumvent confounding and reverse causation (2). In 2 cohorts from the Danish general population, the CCHS (Copenhagen City Heart Study) and the CGPS (Copenhagen General Population Study), we first tested whether LDL-C levels predicted risk of disease observationally. Second, we tested if 11 variants in the *LDLR*, *APOB*, *HMGCR*, *PCSK9*, and *NPC1L1* genes were associated with high LDL-C levels. Third, we tested whether the genetic variants associated with high LDL-C also associated with risk of disease, as an indication of causality. Fourth, we performed instrumental variable analysis to obtain a causal risk estimate per 1-mmol/l (38.6 mg/dl) higher LDL-C level and validated our results in a 2-sample summary-level data MR design with information on lipid levels from the GLGC (Global Lipid Genetics Consortium) and endpoint data from the UK

Biobank. Finally, we performed a meta-analysis to estimate the effect of lowering LDL-C with statin therapy on change in estimated glomerular filtration rate (eGFR).

METHODS

STUDY POPULATIONS. We included 116,419 individuals from 2 similar studies of the Danish general population: the CCHS and the CGPS. Participants were white and of Danish descent, and none were included in >1 study. For further description of the studies and data collection, please see the [Online Appendix](#). Both studies were approved by institutional boards and Danish ethical committees (KF-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 all individuals.

LDL CHOLESTEROL. Total cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured by direct enzymatic methods (Konelab, Thermo Fisher Scientific, Waltham, Massachusetts). Plasma LDL-C levels were calculated using the Friedewald equation if plasma triglyceride levels were ≤ 4.0 mmol/l, and otherwise were measured using a direct assay. LDL-C levels were multiplied by 1.43 in individuals using lipid-lowering drugs, corresponding to an estimated 30% reduction (3). Of the 12,734 individuals receiving lipid-lowering drugs, >97% received statins and 90% had been on therapy for >1 year, indicating that most individuals were chronic statin users. Information on lipid-lowering drug use and years on treatment was self-reported. To examine the observational association between LDL-C and disease, individuals were categorized based on LDL-C levels at baseline, divided at the 50th, 75th, 90th, and 95th percentiles. Individuals with levels below the 50th percentile were defined as the reference group. The cutpoints were selected to reflect a stepwise increase in LDL-C levels from below the median to extreme high values.

GENOTYPES. An ABI PRISM 7900HT Sequence Detection System (Applied Biosystems Inc., Foster City, California) and TaqMan-based assays were used to genotype 11 variants in the *LDLR*, *APOB*, *HMGCR*, *PCSK9*, and *NPC1L1* genes that were previously associated with high LDL-C levels (4): *LDLR* W23X (rs267607213), W66G (rs121908025), and W556S; *APOB* R3500Q (rs5742904); *HMGCR* (rs17238484); *PCSK9* R46L (rs11591147), V474I (rs562556), and E670G (rs505151); and *NPC1L1* (rs17655652, rs41279633, and rs217434). The variants were combined into a weighted allele score, taking the LDL-C-increasing

effect of each allele and its frequency in the population into account (5). The weighted allele score was divided into 5 categories, with cutpoints selected to reflect a stepwise increase in LDL-C levels from below the median to extreme high values.

ENDPOINTS. We performed a prospective cohort study by linking individuals from the CCHS and CGPS to the national Danish Patient Registry and the national Danish Registry of Causes of Death, using each individual's unique national Danish Civil Registration System number. These registries include diagnosis codes according to the World Health Organization's codes of International Classification of Diseases (ICD)-Eighth and Tenth Revisions from all hospital admissions and outpatient clinic visits in Denmark, date of diagnosis, whether the individual is dead or alive, and the date and cause of death. Endpoints were based on diagnoses of retinopathy, peripheral neuropathy, CKD, PAD, and myocardial infarction (MI), and were collected from January 1, 1977, through March 9, 2017. For detailed definitions of endpoints and covariates, please see the [Online Appendix](#).

STATISTICAL ANALYSES. We used Stata SE version 14.2 (StataCorp, College Station, Texas). Deviation from Hardy-Weinberg equilibrium was tested using a Pearson chi-square test. To test for trend across ordered categories of higher LDL-C levels, genotypes, and the weighted gene score, we used the nonparametric Cuzick's extension of a Wilcoxon rank sum test.

To test whether high levels of LDL-C were associated with risk of disease, we used Cox proportional hazards regression with age as time scale and adjusted for sex, birth year, current smoking, body mass index (BMI), hypertension, diabetes mellitus, lipid-lowering drug use, and menopausal status for women to estimate the hazard ratio (HR) for each endpoint.

To test whether the genotypes and the weighted allele score were associated with increased risk of disease, we used unadjusted Cox proportional hazards regression (as genotypes have a constant effect throughout life and are unaffected by confounding factors) with age as time scale. A critical assumption of the MR design is that the genetic instrument should influence risk of disease only through the exposure of interest, that is, LDL-C. To test this, we used logistic regression to assess whether the potential confounders of age, sex, current smoking, BMI, hypertension, diabetes mellitus, and, for women, menopausal status were associated observationally with high LDL-C levels and with the weighted allele score. Instrumental variable analysis was used to

estimate the potential causal association for 1-mmol/l (38.6 mg/dl) higher LDL-C level on risk of disease. Additionally, to validate the results, we conducted 2-sample MR analyses with summary-level data from the GLGC (n = 94,595) and the UK Biobank (n = 408,455). For detailed methods for these analyses, please see the [Online Appendix and Online Table 1](#).

META-ANALYSIS. We performed a meta-analysis investigating the effect of LDL-C reduction by statin treatment on change in eGFR. Randomized controlled trials reporting the effect of statin treatment on eGFR in adult individuals with normal or mild to moderately reduced kidney function (eGFR >30 ml/min/1.73 m²) were included. For description of the methods, search criteria, and details of studies included, please see the [Online Appendix, Online Figure 1, and Online Table 2](#).

RESULTS

Baseline characteristics by LDL-C percentile categories are shown in [Table 1](#). Individuals with LDL-C levels in the 91st to 100th percentile were older; were more often men; were current smokers; had hypertension, diabetes, and higher BMI; and received lipid-lowering drugs more frequently compared with individuals with LDL-C levels below the 50th percentile. Genotype distributions did not deviate from Hardy-Weinberg expectations (all p > 0.12).

OBSERVATIONALLY HIGH LDL-C LEVELS AND RISK OF DISEASE. Baseline mean plasma LDL-C levels were 148% higher in individuals above the 95th percentile (mean 6.7 ± 1.21 mmol/l) compared with individuals at or below the 50th percentile (2.7 ± 0.51 mmol/l) ([Figure 1](#)). Observationally, categories of higher LDL-C levels were not associated with risk of retinopathy (p for trend = 0.12), but with a reduced risk of peripheral neuropathy (p for trend = 0.005), with a multifactorially adjusted HR of 0.67 (95% confidence interval [CI]: 0.51 to 0.87) in individuals with LDL-C levels above the 95th percentile compared to below the 50th percentile. Categories of higher LDL-C levels were associated with a stepwise higher risk of CKD and PAD (p for trend <0.001 for both), with multifactorially adjusted HRs of 1.05 (95% CI: 0.97 to 1.13) and 1.41 (95% CI: 1.23 to 1.62), respectively, in individuals with LDL-C levels above the 95th percentile compared with below the 50th percentile. These results were similar when the categorization of individuals were done without a factor 1.43 correction of LDL-C levels in lipid-lowering drug users and when lipid-lowering drug users were excluded from analyses

TABLE 1 Baseline Characteristics by LDL Cholesterol Percentile Category

	LDL-C Percentiles					p Value for Trend
	0%-50%	51%-75%	76%-90%	91%-95%	96%-100%	
n	59,241 (50)	28,075 (25)	17,462 (15)	5,967 (5)	5,674 (5)	
LDL-C, mmol/l	2.8 (2.4-3.1)	3.7 (3.6-3.9)	4.5 (4.3-4.7)	5.2 (5.1-5.4)	6.3 (5.9-7.1)	
Age, yrs	54 (45-65)	60 (50-68)	61 (53-69)	63 (55-70)	64 (57-70)	<0.001
Women	3,383 (57)	14,691 (52)	9,171 (52)	3,285 (55)	3,194 (56)	<0.001
Menopause (women)	17,915 (53)	11,259 (77)	7,855 (86)	2,987 (91)	2,967 (93)	<0.001
Current smoking	1,061 (18)	5,634 (20)	3,904 (22)	1,452 (24)	1,309 (23)	<0.001
Body mass index, kg/m ²	24.7 (22.5-27.5)	26.0 (23.7-27.5)	26.6 (24.1-29.3)	26.8 (24.5-29.6)	27.0 (24.6-29.8)	<0.001
Total cholesterol, mmol/l	5.0 (4.5-5.5)	6.0 (5.6-6.4)	6.7 (6.2-7.1)	7.3 (5.4-7.8)	6.1 (5.4-8.1)	<0.001
Triglycerides, mmol/l	1.2 (0.9-1.8)	1.5 (1.0-2.1)	1.6 (1.2-2.3)	1.7 (1.3-2.5)	1.8 (1.3-2.5)	<0.001
HDL-C, mmol/l	1.6 (1.3-2.0)	1.5 (1.2-1.8)	1.5 (1.2-1.8)	1.5 (1.2-1.8)	1.5 (1.2-1.8)	<0.001
Glucose, mmol/l	5.1 (4.7-5.6)	5.1 (4.8-5.7)	5.2 (4.8-5.7)	5.2 (4.8-5.7)	5.2 (4.8-5.8)	<0.001
Hypertension	27,061 (46)	16,144 (58)	10,954 (63)	4,094 (69)	4,116 (73)	<0.001
Diabetes mellitus	2,657 (4.5)	1,479 (5.3)	1,067 (6.1)	455 (7.6)	528 (9.3)	<0.001
Lipid-lowering drugs	1,909 (3)	2,406 (9)	2,782 (16)	1,840 (31)	3,909 (69)	<0.001
Antihypertensive drugs*	8,822 (14.9)	5,679 (20.4)	3,993 (22.9)	1,690 (28.4)	2,210 (39.0)	<0.001

Values are n (%) or median (interquartile range). LDL-C concentrations were multiplied by 1.43 in individuals receiving lipid-lowering medication. *We only have information on use of antihypertensive drugs yes/no and not data on the distribution of specific antihypertensive drugs in the Copenhagen cohorts; however, based on nationwide data of the prescriptions of antihypertensive drugs in Denmark from 1995 to 2010, 21% of those on antihypertensive monotherapy received β -blockers, 34% diuretics, 18% calcium-antagonists, 19% angiotensin-converting enzyme inhibitors, and 8% angiotensin receptor blockers (32).

HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.

(Online Figures 2 and 3). The risk of PAD was similar when defined solely by ICD diagnosis codes or solely by ICD procedure codes of lower-extremity revascularization (Online Figure 4). We also performed additional sensitivity analyses excluding individuals on hemodialysis (Online Figure 5) and using a logistic regression model instead of a Cox proportional hazards model (Online Figure 6). These analyses gave similar results as the main analyses. The known stepwise association between higher LDL-C levels and MI is shown as a positive control of study power (p for trend <0.001) (Figure 1).

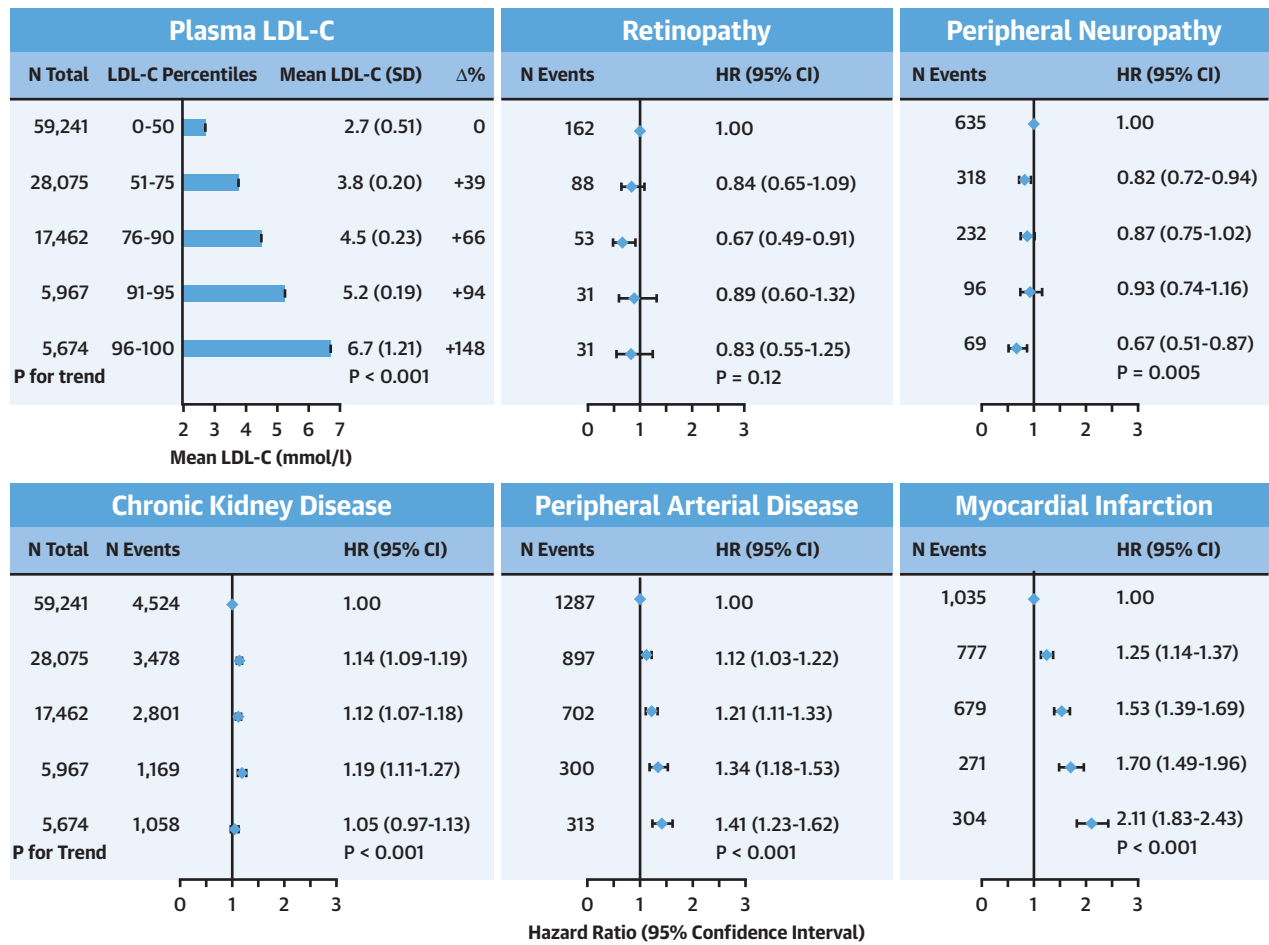
GENETICALLY HIGH LDL-C LEVELS AND RISK OF DISEASE. The selected genetic variants in the *LDLR*, *APOB*, *HMGCR*, *PCSK9*, and *NPC1L1* genes, separately and combined in a weighted allele score, were associated with stepwise higher mean plasma LDL-C levels, with an 18% higher level in the highest allele score category (mean 4.0 ± 1.61 mmol/l) compared with the lowest (3.4 ± 1.11 mmol/l) (Figure 2, Online Figure 7). Categories of genetically higher LDL-C levels were not associated with risk of retinopathy and peripheral neuropathy (p for trend = 0.43 and 0.88), but had a stepwise higher risk of PAD (p = 0.02), with a HR of 1.23 (95% CI: 1.00 to 1.51) in the highest allele score category compared with the lowest (Figure 2). The risk of CKD was higher in individuals in the highest allele score category compared with the lowest, with an HR of 1.22 (95% CI: 1.08 to 1.38). Estimates for the known association

between genetically higher LDL-C levels and MI is shown as a positive control of the genetic instrument (Figure 2).

CONFOUNDING FACTORS. Age, sex, BMI, current smoking status, hypertension, diabetes mellitus, and menopausal status for women were associated with LDL-C levels, but not with the combined genetic variants, indicating that pleiotropic effects through any of the above factors are unlikely (Online Figure 8).

CAUSAL ASSOCIATION BETWEEN HIGH LDL-C LEVELS AND DISEASE. We found no observational or causal association between LDL-C levels and risk of retinopathy (p = 0.10 and 0.94) (Figure 3). There was an observationally lower risk of peripheral neuropathy with higher LDL-C levels (HR: 0.92; 95% CI: 0.88 to 0.97; per 1-mmol/l [38.6 mg/dl] higher LDL-C level), but no causal association (p = 0.86). Observationally, high LDL-C levels were associated with high risk of CKD based on ICD diagnoses and eGFR <60 ml/min/1.73 m² combined (HR: 1.04; 95% CI: 1.02 to 1.06), and when based on eGFR <60 ml/min/1.73 m² alone (odds ratio: 1.24; 95% CI: 1.22 to 1.27) per 1-mmol/l higher LDL-C level. In the causal analyses, the risk ratio for a 1-mmol/l (38.6 mg/dl) higher LDL-C level was 3.83 (95% CI: 2.00 to 7.34) for CKD based on ICD codes and eGFR <60 ml/min/1.73 m² combined, and 3.81 (95% CI: 2.03 to 7.15) for eGFR <60 ml/min/1.73 m² alone. High LDL-C levels were

FIGURE 1 Risk of Retinopathy, Peripheral Neuropathy, Chronic Kidney Disease, PAD, and Myocardial Infarction as a Function of LDL-C Percentile Categories



(Top left) Mean low-density lipoprotein cholesterol (LDL-C) level as a function of percentile categories, and prospective risk of (top middle) retinopathy, (top right) peripheral neuropathy, and (bottom) chronic kidney disease, peripheral arterial disease (PAD), and myocardial infarction as a function of LDL-C percentile categories. Chronic kidney disease was defined as a composite of International Classification of Diseases (ICD) codes and/or estimated glomerular filtration rate <60 ml/min/1.73 m². An event of PAD was defined as either an ICD code for PAD or a procedural code for lower extremity revascularization, whichever came first. Risk of myocardial infarction as a function of LDL-C level percentile categories is shown as a positive control of study power. Estimates are adjusted for sex, birth year, current smoking, body mass index, hypertension, diabetes mellitus, lipid-lowering drug use, and menopause for women. Individuals with an event before baseline were excluded. Δ% = percent difference in LDL-C; CI = confidence interval; HR = hazard ratio.

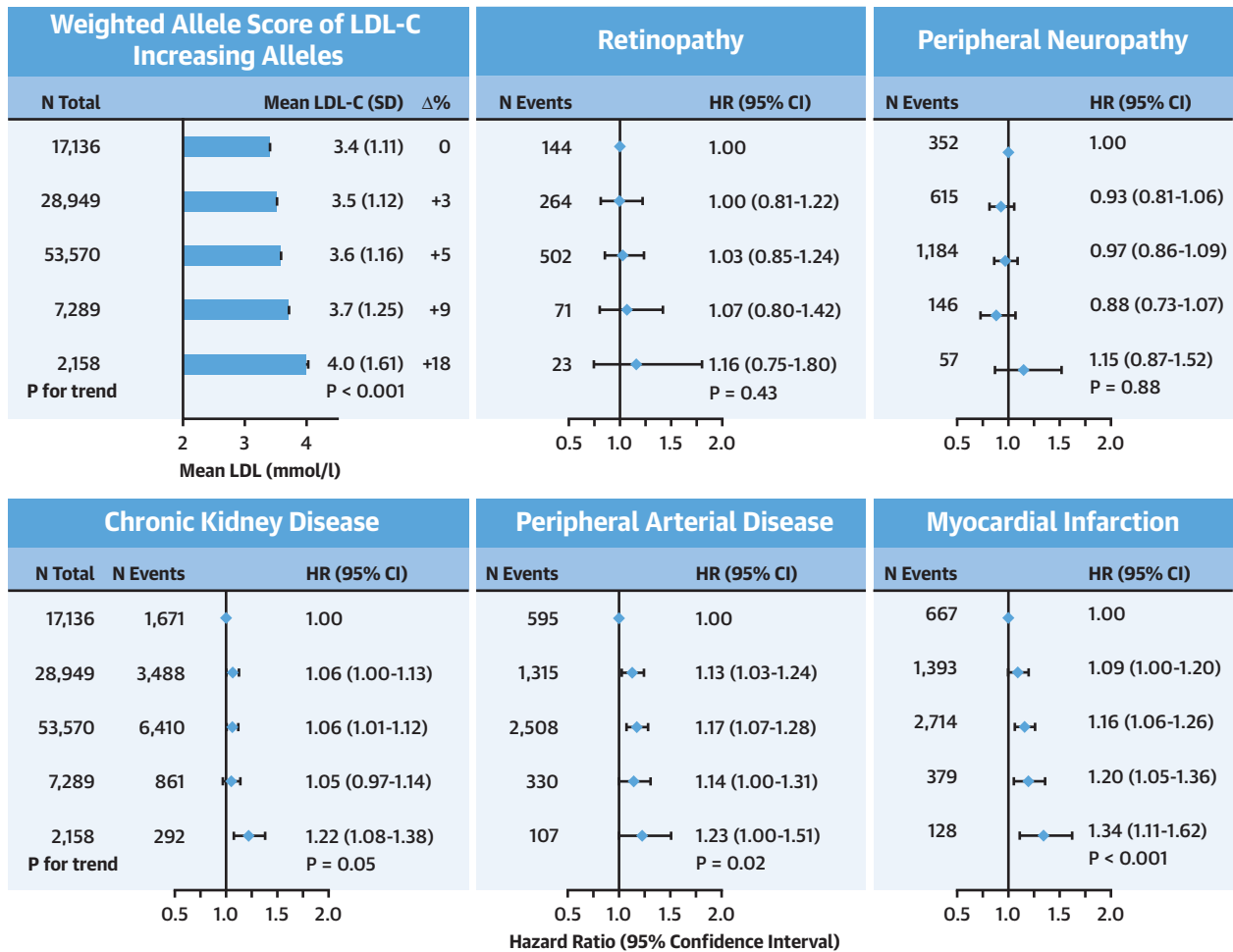
associated with high risk of PAD both observationally and causally, with an HR of 1.09 (95% CI: 1.05 to 1.12) and a risk ratio of 2.09 (95% CI: 1.30 to 2.38) for a 1 mmol/l (38.6 mg/dl) higher LDL-C level, respectively.

Using summary level data for 45 genetic variants associated with high LDL-C levels in the GLGC and combined with endpoint data from the UK Biobank into a causal estimate by 2-sample MR regression, the inverse variance weighted estimates showed similar results for retinopathy (p = 0.56), peripheral neuropathy (p = 0.77), and PAD (risk ratio: 1.76; 95% CI:

1.22 to 2.54; p = 0.002) but not for CKD based on ICD codes (risk ratio: 0.93; 95% CI: 0.77 to 1.12; p = 0.21) (Figure 3). The corresponding 2-sample MR estimates using MR Egger and weighted median regression showed comparable results, and with no indication of pleiotropy (all p values for the MR Egger intercept >0.13) (Online Figure 9).

EFFECT OF LOWERING LDL-C LEVELS ON ESTIMATED GLOMERULAR FILTRATION RATE. To further clarify the associations between LDL-C levels and kidney function, we performed a meta-analysis combining

FIGURE 2 Risk of Retinopathy, Peripheral Neuropathy, Chronic Kidney Disease, PAD, and Myocardial Infarction as a Function of Increasing Allele Score Categories



(Top left) Mean LDL-C level as a function of weighted allele score in categories, and prospective risk of (top middle) retinopathy, (top right) peripheral neuropathy, and (bottom) chronic kidney disease, PAD, and myocardial infarction as a function of increasing allele score categories. Risk of myocardial infarction is shown as a positive control of the genetic instrument. Estimates are unadjusted as an individual's genotype is largely constant throughout life, and is unlikely to be affected by confounding factors. Individuals with an event before baseline were excluded. Abbreviations as in Figure 1.

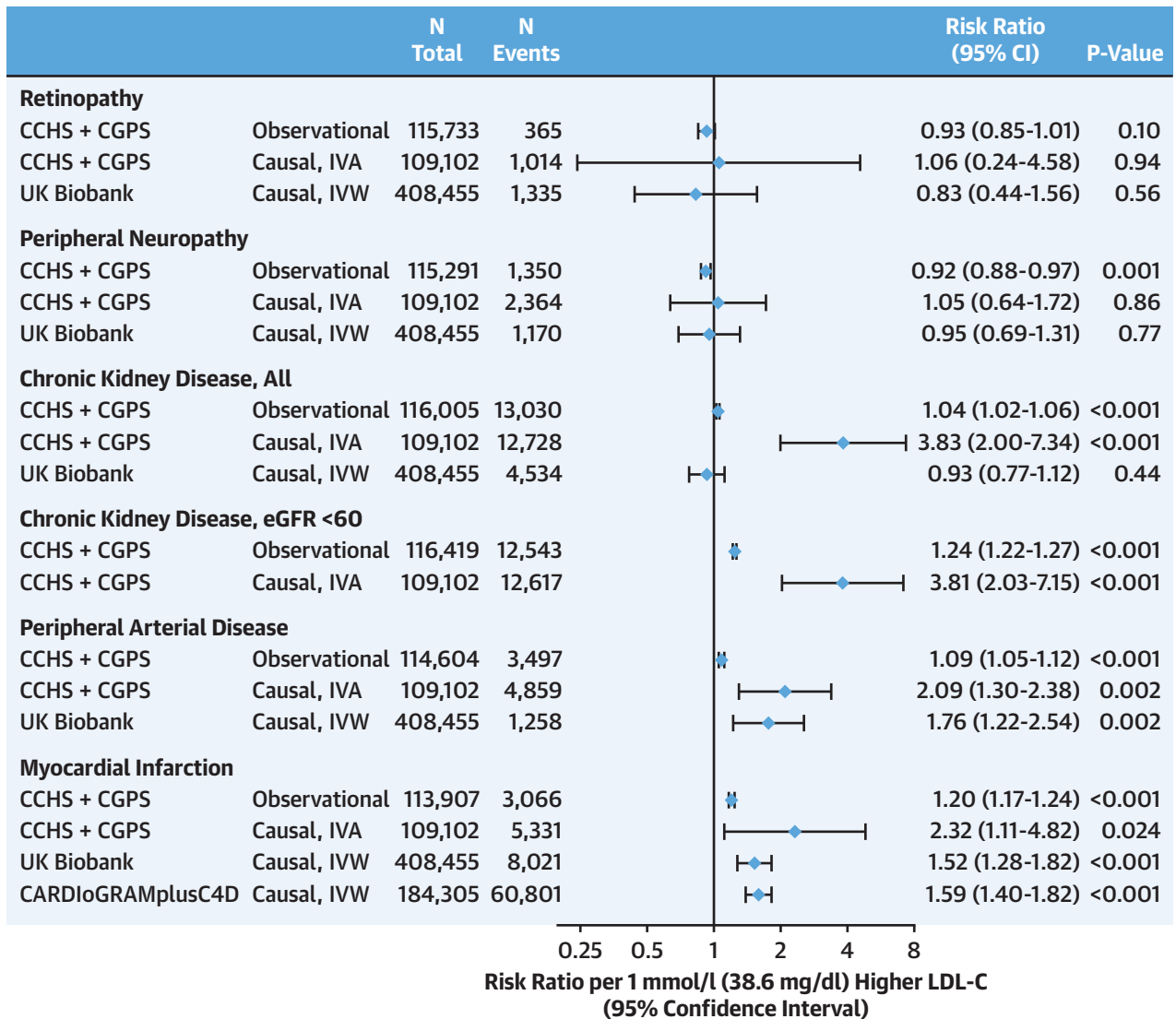
13 randomized controlled trials (n = 64,134), examining effect of statin therapy on change in eGFR. The mean treatment time was 3.7 years, and the weighted mean LDL-C at the end of the trial was 0.85 mmol/l lower in statin treated individuals compared with control subjects. For a 1-mmol/l (38.6 mg/dl) reduction in LDL-C levels, the random effects weighted mean eGFR improvement was 2.69 ml/min/1.73 m² (95% CI: 1.48 to 3.94 ml/min/1.73 m²) in statin-treated individuals compared with control subjects at the end of treatment (Figure 4). Correspondingly, a 1-mmol/l (38.6 mg/dl) observationally lower LDL-C level in the CCHS and CGPS was associated with a

1.95 ml/min/1.73 m² (95% CI: 1.88 to 2.02 ml/min/1.73 m²) higher mean eGFR, and a 1-mmol/l (38.6 mg/dl) lifelong genetically lower LDL-C level was causally associated with a 5.92 ml/min/1.73 m² (95% CI: 4.97 to 6.86 ml/min/1.73 m²) higher mean eGFR.

DISCUSSION

In 116,419 individuals from the Copenhagen population, we found no observational or causal association between high LDL-C levels and high risk of retinopathy and peripheral neuropathy; however, we found

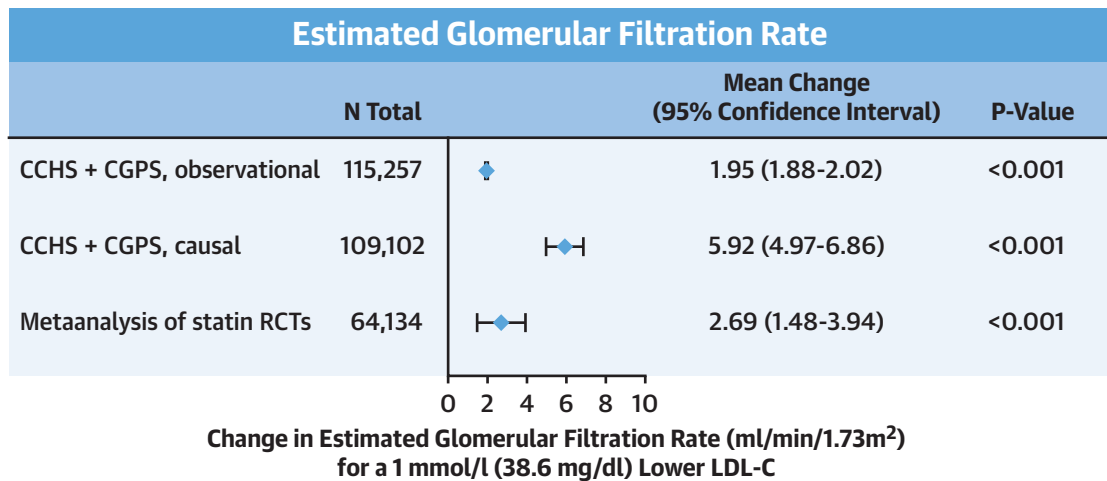
FIGURE 3 Risk of Retinopathy, Peripheral Neuropathy, Chronic Kidney Disease, PAD, and Myocardial Infarction per 1-mmol/l (38.6 mg/dl) Higher Observational and Causal LDL-C Level



Chronic kidney disease is presented as both defined by ICD codes and estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m² combined and by eGFR <60 ml/min/1.73 m² alone. Hazard ratios for a 1-mmol/l higher observational LDL-C level were estimated using Cox regression and risk ratios for genetically higher LDL-C levels were derived from instrumental variable analyses (IVA). The strength of the genetic instrument was confirmed by F statistics for the weighted allele score of 198, explaining 1% of the variation in LDL-C levels. Risk was also estimated using summary-level data from the UK Biobank for 45 genetic variants associated with LDL-C levels in the Global Lipid Genetics Consortium, combined by inverse-variance weighted (IVW) regression. CCHS = Copenhagen City Heart Study; CGPS = Copenhagen General Population Study; other abbreviations as in Figure 1.

an observational and causal association between high LDL-C levels and high risk of CKD and PAD (Central Illustration). The findings were replicated with similar results using summary level data from the GLGC and the UK Biobank for retinopathy, peripheral neuropathy, and PAD, but not for CKD based on ICD codes. In a meta-analysis of 13 randomized controlled

statin trials, reduction of LDL-C levels resulted in improved mean eGFR at end of treatment, with effect estimates similar to the observational and genetic, causal estimates from the Copenhagen studies. Overall, the findings were consistent across genotypes and for both the targeted conventional and nontargeted 2-sample MR approach, indicating that

FIGURE 4 Change in eGFR for a 1-mmol/l (38.6 mg/dl) Lower Observational, Causal and Statin-Treated LDL-C Level

The observational estimate was calculated by linear regression. The genetic estimate was derived from instrumental variable analyses. The meta-analysis estimate is the random effects weighted mean difference between the treatment group and the control group in randomized controlled statin trials (RCTs) at end of treatment. Abbreviations as in [Figures 1 and 3](#).

the results are representative for LDL-C levels per se and not reflecting a single LDL-C pathway, represented by the selected genetic variants.

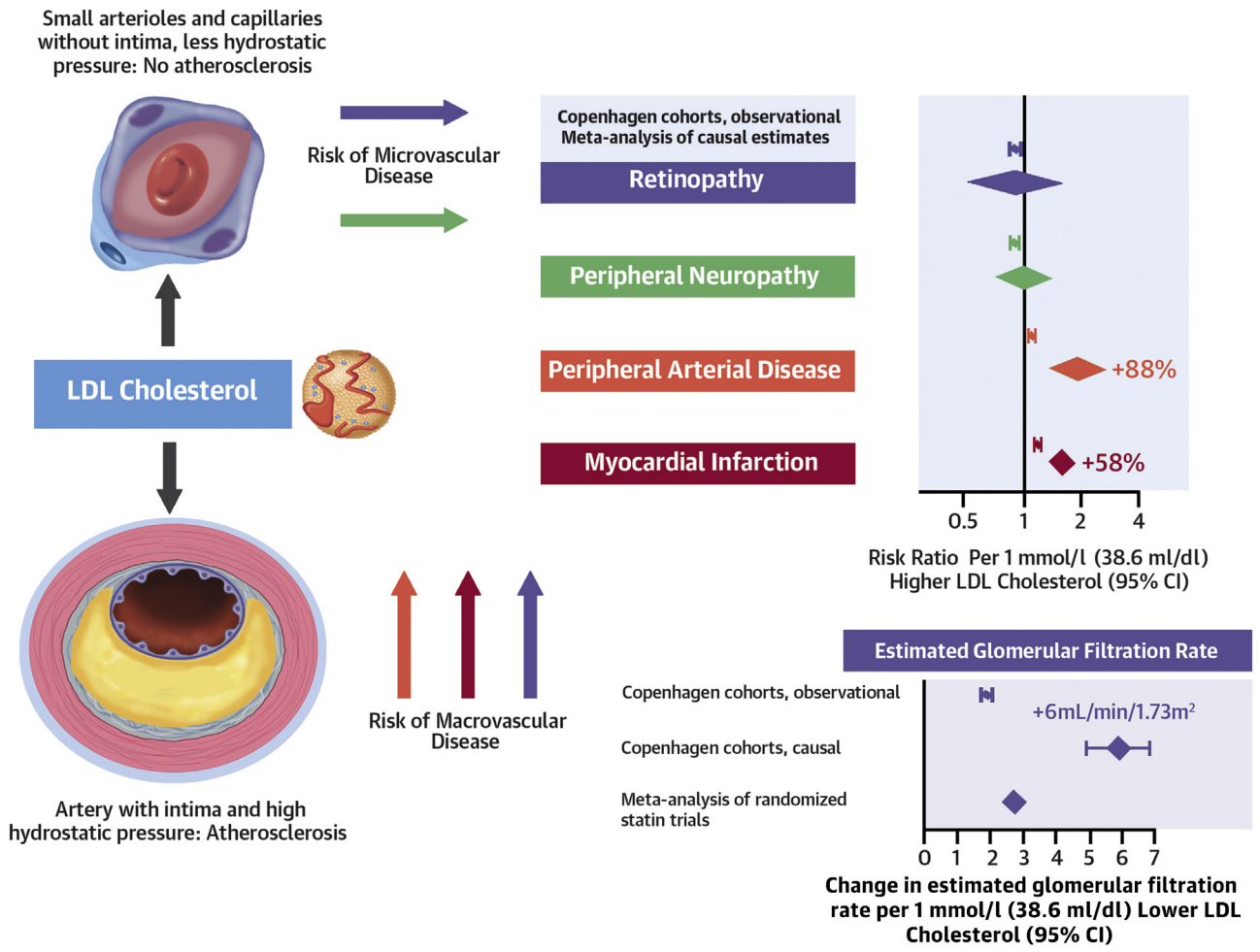
Several studies have assessed the association between LDL-C levels and retinopathy and the effect of statin treatment on progression of retinopathy with inconsistent findings (6-8). In a randomized controlled trial including 2,838 patients with type 2 diabetes, atorvastatin treatment did not influence retinopathy progression (8). Fenofibrate has been shown to have a preventive effect on progression of retinopathy compared with placebo and with statin treatment alone; however, this effect was not accompanied by changes in LDL-C levels (7,8). Together with the present findings, this suggests that the preventive effect of fenofibrate on retinopathy progression is not due to LDL-C lowering, but is mediated through other fenofibrate-induced mechanisms.

The most common etiological cause of peripheral neuropathy is diabetes, and except for glucose-lowering drugs, no evidence-based treatments are available (9). Neuropathy remains idiopathic in around 30% of cases, and even though intensive glycemic control improves the disease substantially in type 1 diabetes, a residual risk is still present, and in meta-analyses, the effect of glucose-lowering drugs in type 2 diabetic neuropathy do not reach statistical significance (10). Obesity and components of the metabolic syndrome have been identified as

independent risk factors of peripheral neuropathy also in the normoglycemic state (11). Further studies are needed to elucidate potential causal pathways.

Experimental and observational studies have found associations between dyslipidemia and CKD, which biologically may be explained both by macrovascular changes in the renal arteries as well as microvascular changes in the glomeruli (12-14). A recent 2-sample MR study based on case/control summary-level data found no causal association between LDL-C levels and CKD, and only a small positive causal association between higher LDL-C and eGFR in univariate analysis (15). Compared with the Copenhagen cohorts, individuals included in this summary-level data study were more heterogenous in terms of ethnicity, progression of CKD, and comorbidity status, and generally had a higher prevalence of diabetes and hypertension (16). Furthermore, the availability of individual-level phenotype and genotype data in the Copenhagen studies made it possible to use a weighted allele score, which reflects the combined effect of the included genetic variants on circulating LDL-C levels and increases study power (5). In meta-analyses of clinical intervention trials, lipid-lowering drugs did not reduce the risk of kidney failure, but consistent with our data, showed a modest reduction in the rate of eGFR decline in individuals with a varying range of CKD not requiring dialysis (17,18). Interestingly, some of these studies found a larger effect on eGFR for high-intensity statin

CENTRAL ILLUSTRATION Impact of Low-Density Lipoprotein Cholesterol on Microvascular Versus Macrovascular Disease



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Potential mechanisms, observational associations, and causal effects in a general population setting. LDL = low-density lipoprotein.

treatment, suggesting a dose-dependent effect. Future intervention trials evaluating the effect of PCSK9 inhibition on kidney function may further elucidate this potential dose-dependent effect.

As for CKD, current guidelines recommend statin use in all individuals with PAD due to their high risk of cardiovascular events (19). The evidence supporting an effect of lipid-lowering drugs on PAD progression per se is limited, and it has been suggested that LDL-C levels are a less important risk factor for PAD than for cardiovascular disease in general (20). However, an effect on peripheral vascular events has been shown both for statins and, lately, PCSK9 inhibitors (21-23), and registry studies

suggest that individuals with PAD are heavily undertreated (23).

The present results suggest a causal involvement of LDL-C in the pathogenesis of CKD and PAD, which are mixed vascular diseases involving both large and small vessels, and no causal involvement in the pathogenesis of retinopathy and peripheral neuropathy, which may be classified as pure microvascular diseases. A plausible biological explanation might be that an artery requires a certain size and certain morphological characteristics (i.e., an artery intima, elastic lamina, and media) for lipoproteins to accumulate and for the atherosclerotic process to be initiated (24). Physiological stimuli such as blood flow

and wall tension are also important determinants of atherosclerosis progression, stimuli that are attenuated in microvascular beds.

STUDY LIMITATIONS. A potential limitation of the MR approach is that the selected genetic variants, apart from being associated with high LDL-C levels, are also associated with confounders of the exposure-outcome association (25). We did not find any associations among the genetic instruments and age, sex, BMI, smoking, hypertension, diabetes mellitus, or, for women, menopause. Also, although our aim was to study the general population, the confirmation cohorts used in the 2-sample MR consisted of selected samples that may not be representative of the general population and may thus not be completely comparable. In the UK Biobank, the participation rate was low (participation rate CCHS: 50%, CGPS: 45%, and the UK Biobank: 5%) (26), and healthy participant bias may explain some of the differences in prevalence of and risk estimates for CKD between the CCHS + CGPS and the UK Biobank, along with the substantial differences in follow-up time (up to 40 years in the CCHS + CGPS compared with up to 5 years in the UK Biobank). Another limitation is that eGFR was calculated from a single measurement of plasma creatinine, which may lead to some misclassification of kidney disease. However, the prevalence of eGFR <60 ml/min/1.73 m² in the CCHS + CGPS was approximately 10%, which corresponds well with prevalence estimates from other general populations (27). In observational analyses, we multiplied LDL-C levels with a correction factor of 1.43 in individuals receiving lipid-lowering drugs (corresponding to an average 30% LDL-C reduction by statins) before categorizing them based on LDL-C levels (3), to better capture the LDL-C levels in these individuals throughout most of their lives. We acknowledge that a single correction factor for all individuals receiving lipid-lowering drugs is an oversimplification that does not take type, dose, and time of treatment into account. However, we also believe that not correcting for lipid-lowering drug use would misclassify individuals according to their true vascular risk.

Strengths of the study include examination of a large number of individuals from a genetically homogenous general population, access to individual participant data of high validity, and no losses to follow-up. The MR approach allows us to examine causal effects, minimizing confounding and reverse causation. The similar results found using the 2-sample MR approach with summary data from the GLGC and the UK Biobank increases the generalizability of the genetic instrument (to represent LDL-C

per se and not a single pathway) as well as the validity of the results by being reproducible in another population. We performed 2 sensitivity analyses in the 2-sample MR approach: MR Egger adjusting for directional pleiotropy, and weighted median estimates regression accounting for up to 50% of information coming from invalid or weak instruments (28,29); the results were in the same direction and there was no indication of pleiotropy, confirming the validity of the instrument (28,29). The results from the conventional and the 2-sample MR approach were consistent, except for CKD. We therefore performed a meta-analysis of randomized controlled statin trials evaluating the effect of LDL-C-lowering on eGFR and found estimates similar to those from the Copenhagen studies, supporting a causal association but with a modest effect size on eGFR.

Our findings show that high LDL-C levels are not causally associated with high risk of retinopathy and peripheral neuropathy, suggesting that lowering of LDL-C levels has no effect in prevention of these diseases. In contrast, high LDL-C levels were observationally and genetically associated with high risk of CKD and PAD, suggesting that LDL-C is causally related to risk of CKD and PAD, and supporting a role of LDL-C-lowering drugs in the prevention of these diseases. In current guidelines, onset of PAD or eGFR <60 ml/min/1.73 m² is used as a cut-off for recommended intervention with lipid-lowering therapy (19,30,31). Our findings suggest that individuals at risk for PAD and CKD may benefit from LDL lowering at an even earlier stage, that is, before disease onset. However, the predicted effect of lowering LDL-C on eGFR was modest, and other risk factors such as hypertension and hyperglycemia are probably more important determinants of CKD progression. For PAD, the causal risk estimates were comparable to the causal risk estimates for MI, which highlights the importance of LDL-C-lowering treatment with a focus on lowering the risk of peripheral vascular events as well as cardiovascular events.

CONCLUSIONS

High LDL-C levels were not causally associated with high risk of retinopathy and peripheral neuropathy; however, high LDL-C levels were observationally and genetically associated with high risk of PAD and CKD, suggesting that LDL-C is causally involved in the pathogenesis of these diseases. Although genetic findings for CKD in the Copenhagen studies were not confirmed in the UK Biobank, statin trials concurred with the Copenhagen studies that lower LDL-C is causally related with better kidney function.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: High levels of LDL-C play a causal role in coronary artery disease and PAD but have no proven relationship to microvascular diseases, such as retinopathy and neuropathy.

TRANSLATIONAL OUTLOOK: Because high LDL-C levels are genetically associated with a high risk of CKD and certain forms of vascular disease, future studies should seek to ascertain the range of microvascular diseases that could be prevented by lowering LDL-C.

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KEY WORDS chronic kidney disease, LDL-C, Mendelian randomization, meta-analysis, neuropathy, peripheral arterial disease, retinopathy

APPENDIX For an expanded Methods section as well as supplemental tables and figures, please see the online version of this paper.