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Association Between Low-Density Lipoprotein Cholesterol–Lowering Genetic Variants and Risk of Type 2 Diabetes
A Meta-analysis

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IMPORTANCE Low-density lipoprotein cholesterol (LDL-C)–lowering alleles in or near *NPC1L1* or *HMGCR*, encoding the respective molecular targets of ezetimibe and statins, have previously been used as proxies to study the efficacy of these lipid-lowering drugs. Alleles near *HMGCR* are associated with a higher risk of type 2 diabetes, similar to the increased incidence of new-onset diabetes associated with statin treatment in randomized clinical trials. It is unknown whether alleles near *NPC1L1* are associated with the risk of type 2 diabetes.

OBJECTIVE To investigate whether LDL-C-lowering alleles in or near *NPC1L1* and other genes encoding current or prospective molecular targets of lipid-lowering therapy (ie, *HMGCR, PCSK9, ABCG5/G8, LDLR*) are associated with the risk of type 2 diabetes.

DESIGN, SETTING, AND PARTICIPANTS The associations with type 2 diabetes and coronary artery disease of LDL-C-lowering genetic variants were investigated in meta-analyses of genetic association studies. Meta-analyses included 50 775 individuals with type 2 diabetes and 270 269 controls and 60 801 individuals with coronary artery disease and 123 504 controls. Data collection took place in Europe and the United States between 1991 and 2016.

EXPOSURES Low-density lipoprotein cholesterol–lowering alleles in or near *NPC1L1, HMGCR, PCSK9, ABCG5/G8,* and *LDLR*.

MAIN OUTCOMES AND MEASURES Odds ratios (ORs) for type 2 diabetes and coronary artery disease.

RESULTS Low-density lipoprotein cholesterol–lowering genetic variants at *NPC1L1* were inversely associated with coronary artery disease (OR for a genetically predicted 1-mmol/L [38.7-mg/dL] reduction in LDL-C of 0.61 [95% CI, 0.42-0.88]; $P = .008$) and directly associated with type 2 diabetes (OR for a genetically predicted 1-mmol/L reduction in LDL-C of 2.42 [95% CI, 1.70-3.43]; $P < .001$). For *PCSK9* genetic variants, the OR for type 2 diabetes per 1-mmol/L genetically predicted reduction in LDL-C was 1.19 (95% CI, 1.02-1.38; $P = .03$). For a given reduction in LDL-C, genetic variants were associated with a similar reduction in coronary artery disease risk ($I^2 = 0\%$ for heterogeneity in genetic associations; $P = .93$). However, associations with type 2 diabetes were heterogeneous ($I^2 = 77.2\%$; $P = .002$), indicating gene-specific associations with metabolic risk of LDL-C-lowering alleles.

CONCLUSIONS AND RELEVANCE In this meta-analysis, exposure to LDL-C-lowering genetic variants in or near *NPC1L1* and other genes was associated with a higher risk of type 2 diabetes. These data provide insights into potential adverse effects of LDL-C-lowering therapy.

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Supplemental content

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Treatment with statins, the pharmacological agents of choice for low-density lipoprotein cholesterol (LDL-C)–lowering therapy in cardiovascular prevention,[1,2] is associated with weight gain and a higher incidence of new-onset type 2 diabetes.[3-5] Ezetimibe, an inhibitor of the LDL-C transporter Niemann-Pick C1-Like 1 (NPC1L1),[6,7] has been approved as a lipid-lowering agent, but it is unclear whether its use will be associated with an adverse metabolic risk profile.

There is considerable interest in predicting the efficacy and safety of therapeutic targets early in the drug development process. Drug targets with supporting human genetic evidence have been shown to have lower attrition rates during drug development.[8] Variation in genes encoding drug targets has been used to predict both the efficacy and safety of pharmacological perturbation of those targets.[9,10] In particular, LDL-C-lowering alleles in *HMGCR*,[5,11] which encodes the molecular target of statins, have been successfully used as genetic proxies to study the effects of these drugs.[5,11] Furthermore, LDL-C-lowering alleles in *HMGCR* are associated with higher risk of type 2 diabetes and higher body mass index (BMI) in genetic studies,[5] similar to the safety profile of statins in meta-analyses of randomized clinical trials (RCTs).[3-5]

The efficacy of adding ezetimibe to simvastatin in secondary cardiovascular prevention was supported by the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT).[6,7] Immediately before and after the publication of the trial results, studies were reported describing the use of genetic variants at *NPC1L1* to predict the efficacy of NPC1L1 inhibition in the prevention of coronary events.[11,12] The purpose of this study was to use naturally occurring LDL-C-lowering alleles at *NPC1L1* to investigate the potential associations between NPC1L1 inhibition and the risk of type 2 diabetes. Alleles that lower LDL-C in or near genes encoding other current or prospective molecular targets of LDL-C-lowering therapy were studied.

Key Points

Question Are low-density lipoprotein cholesterol (LDL-C)–lowering alleles at *NPC1L1* or other genes associated with the risk of type 2 diabetes?

Findings In a meta-analysis of genetic association studies including 50 775 individuals with type 2 diabetes and 270 269 controls, LDL-C-lowering polymorphisms at *NPC1L1* were associated with a statistically significant odds ratio of 2.42 for type 2 diabetes per genetically predicted reduction of 1 mmol/L (38.7 mg/dL) in LDL-C. Low-density lipoprotein cholesterol–lowering polymorphisms at *HMGCR* and *PCSK9* were associated with a higher risk of diabetes.

Meaning These data provide insights into potential adverse effects of LDL-C-lowering therapy.

Methods

Study Design

The association of LDL-C-lowering polymorphisms near *NPC1L1* with the risk of type 2 diabetes was investigated in meta-analyses of genetic association studies. The associations of LDL-C-lowering alleles in or near genes encoding other current or prospective molecular targets of LDL-C-lowering therapy[11] (ie, *HMGCR, PCSK9, ABCG5/G8, LDLR*) with type 2 diabetes, coronary artery disease, and continuous cardiometabolic traits were studied. A summary of the studies included in each analysis appears in eTable 1 in the Supplement.

Participants

The association of LDL-C-lowering alleles with type 2 diabetes was estimated in a meta-analysis of 50 775 individuals with type 2 diabetes and 270 269 controls from the European Prospective Investigation into Cancer and Nutrition (EPIC)-InterAct study (a case-cohort study nested within the EPIC study, which was a cohort study of 500 000 European participants followed-up for an average of 8 years),[13] the UK Biobank study,[14] and the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM).[15] An additional 11 studies (4496 cases and 50 677 controls) previously reported by Swerdlow et al[5] were included in the analyses of the association with type 2 diabetes of rs12916 in *HMGCR* (eFigure 1 in the Supplement). The combined association of *NPC1L1* genetic variants in subgroups by age, sex, and BMI was analyzed in 14 657 unrelated cases of type 2 diabetes and 118 854 controls from the EPIC-InterAct study and the UK Biobank study with available individual-level genotyping data.

EPIC-InterAct

Eight of the 10 constituent EPIC cohorts agreed to take part in the EPIC-InterAct study, leaving 455 680 participants for screening. Individuals were excluded from EPIC-InterAct if they did not have stored blood (n = 109 625) or information on diabetes status (n = 5821; 1.3% of participants screened for inclusion). From the remaining 340 234 participants, 12 403 individuals who developed type 2 diabetes during follow-up constituted the incident case group of EPIC-InterAct and a random group of 16 154 individuals free of diabetes at baseline constituted the subcohort group of EPIC-InterAct.[13] Subcohort participants were previously shown to be representative of eligible EPIC participants within each country.[13] Data on 20 831 participants with available genotyping (with no overlap with DIAGRAM[15]) were included in the main analysis. Data on the 22 494 participants (including participants overlapping with DIAGRAM) with available genotyping were included in subgroup analyses. Type 2 diabetes status was available for all participants. Individuals without genotype data were excluded from the study. Data collection took place between 1991 and 2016. Participant characteristics and genotyping methods have been previously reported in detail[13] and are summarized in **Table 1** and eTable 2 in the Supplement.

The UK Biobank Study

The UK Biobank study is a population-based cohort of 500 000 people aged 40 to 69 years who were recruited from

Table 1. Participants of the EPIC-InterAct Study, the UK Biobank Study, and DIAGRAM

	EPIC-InterAct Study Countries: Multiple in Europe Type of Genotyping Chip: Illumina 660w Quad and Illumina CoreExome Chip Imputation Panel: Haplotype Reference Consortium		UK Biobank Study Countries: United Kingdom Type of Genotyping Chip: Affymetrix UK Biobank Axiom Array Imputation Panel: 1000 Genomes Phase 3 Plus UK10K		DIAGRAM Countries: Europe and United States[a] Type of Genotyping Chip: Multiple[b] Imputation Panel: HapMap	
	Type 2 Diabetes	Controls	Type 2 Diabetes	Controls	Type 2 Diabetes	Controls
No. of participants	10 071[c]	12 423[c]	6627	143 765	34 840	114 981
Age, mean (SD), y	56 (8)	52 (9)	60 (7)	56 (8)	59 (10)	54 (14)
Female sex, No. (%)	5037 (50)	7713 (62)	2349 (35)	77 397 (54)	14 621 (42)	60 377 (53)
Current smokers, No. (%)	2830 (28)	3240 (26)	811 (12)	18 149 (13)	NA	NA
Body mass index, mean (SD)[d]	29.7 (4.8)	25.8 (4.1)	31.9 (5.9)	27.3 (4.7)	29.7 (5.9)	26.5 (4.5)
Waist-to-hip ratio	0.92 (0.09)	0.85 (0.09)	0.95 (0.08)	0.87 (0.09)	NA	NA
Blood pressure, mean (SD), mm Hg						
Systolic	144 (20)	132 (19)	141 (17)	138 (19)	NA	NA
Diastolic	87 (11)	82 (11)	82 (10)	82 (10)	NA	NA
Cholesterol, mean (SD), mmol/L						
Low-density lipoprotein	4.0 (1)	3.8 (1)	NA[e]	NA[e]	NA	NA
High-density lipoprotein	1.2 (0.4)	1.5 (0.4)	NA[e]	NA[e]	NA	NA
Triglycerides, median (IQR), mmol/L	1.7 (1.2-2.5)	1.1 (0.8-1.6)	NA[e]	NA[e]	NA	NA

Abbreviations: DIAGRAM, DIAbetes Genetics Replication And Meta-analysis; EPIC, European Prospective Investigation into Cancer and Nutrition; IQR, interquartile range; NA, not available.

To convert high-density and low-density lipoprotein cholesterol to mg/dL, divide by 0.0259.

[a] There was a small South Asian component of the study, which accounts for 2.44% of participants.

[b] Chips included Affymetrix Human SNP Array 6.0; Illumina HumanHap 300, 300/370, and 550; Affymetrix Genechip 500K and MIPS 50K; and the Cardio-Metabolchip.

[c] There were 9308 cases of type 2 diabetes and 11 523 controls included in the main analysis of the association of genetic variants with type 2 diabetes after the exclusion of participants overlapping with DIAGRAM.

[d] Calculated as weight in kilograms divided by height in meters squared.

[e] Measurement of blood lipid concentrations is ongoing. The data release is planned for the end of 2016.

2006 through 2010 from several centers across the United Kingdom.[14] The association of genetic variants with prevalent type 2 diabetes was estimated in 6627 cases and 143 765 controls of the UK Biobank study data set who had available genotype data. Genotyping was attempted in 152 770 individuals and failed in only 480 instances (0.3%). Among a total of 152 290 participants with available genotype data, type 2 diabetes status was adjudicated in 150 392 (98.8%) participants. Type 2 diabetes was defined on the basis of self-reported physician diagnosis at nurse interview or digital questionnaire, age at diagnosis older than 36 years, and use of oral medications for diabetes. Data collection took place between 2006 and 2016. Participant characteristics and genotyping information appear in Table 1 and eTable 2 in the Supplement.

DIAGRAM

DIAGRAM is a research consortium that published the largest meta-analysis of genome-wide association studies for type 2 diabetes in individuals of European descent.[15] Type 2 diabetes association results were made publicly available for up to 34 840 cases and 114 981 controls from 38 genetic association studies with a case-control or cohort design.[15] Fifty percent of the participants were women and the average age was 55 years.[15] Imputation was performed using the HapMap reference panel.[15] Participant exclusion criteria encompassed duplicate samples, relatedness, mismatch between self-reported and genotype-determined sex, outlying heterozygosity and non-European descent. Type 2 diabetes status was available in all participants. Data collection took place between 2002 and 2012. Participant characteristics are reported in Table 1 and further characteristics of the studies included in the DIAGRAM meta-analysis were reported previously.[15]

The likelihood of bias for studies participating in this meta-analysis was deemed low on the basis of (1) the low proportion of participants with missing data on exposure or outcome, (2) the high-quality genotyping or imputation of genetic variants included in the study (eTable 2 in the Supplement), (3) the low likelihood of bias by case status in genotyping errors or genotype misclassification, (4) the consideration that if any nondifferential misclassification of exposure or outcome occurred, it would result in a bias toward the null, and (5) the consideration that genetic variants are less likely to be affected by confounding or reverse causality.[16,17] On this basis, studies were deemed suitable for pooling by meta-analysis.

Pooling of Other Data

For the genetic variants included in these analyses, LDL-C association estimates were obtained from genetic association results in up to 188 577 participants of the Global Lipids Genetics Consortium.[18] In addition to type 2 diabetes, the association of these LDL-C-lowering alleles with coronary artery disease and continuous cardiometabolic traits was estimated in large meta-analyses of genome-wide association

studies. For coronary artery disease, data were from the Coronary ARtery DIsease Genome wide Replication and Meta-analysis (CARDIoGRAM) plus the Coronary Artery Disease (C4D) Genetics (CARDIoGRAMplusC4D) Consortium meta-analysis (60 801 cases and 123 504 controls).[19] For glycemic traits, including fasting glucose[20,21] (n = 133 010), glucose level 2 hours after an oral glucose challenge[20,22] (n = 42 854) and fasting insulin levels[20,21] (natural-logarithm transformed; n = 108 557), data were from the Meta-Analysis of Glucose and Insulin-related traits Consortium (MAGIC).[20-22] For anthropometric traits, including BMI (n = 333 495) and waist-to-hip ratio (n = 224 047), data were from the Genetic Investigation of ANthropometric Traits (GIANT) Consortium.[23,24] Details appear in eTable 1 in the Supplement.

In exploratory analyses, the burden of protein-truncating and probably deleterious missense variants in *NPC1L1*, *HMGCR*, *PCSK9*, *ABCG5*, *ABCG8* and *LDLR* was estimated from exome sequencing studies of 8373 cases with type 2 diabetes and 8466 controls (Type 2 Diabetes Multi-Ethnic Sequencing [T2D-GENES] Consortium, Genetics of Type 2 Diabetes [GoT2D] Consortium, DIAGRAM Consortium. May 26, 2016; http://www.type2diabetesgenetics.org/).

Selection of Genetic Variants

The combined association of 2 LDL-C-lowering genetic variants near *NPC1L1* with type 2 diabetes constituted the primary analysis of the study (**Table 2**). These variants were identified as having distinct effects on LDL-C levels in approximate conditional analyses using the GCTA software[25,26] (description appears below and in eFigure 2 in the Supplement). In sensitivity analyses, the combined association of 5 LDL-C-lowering alleles near *NPC1L1* (previously used to predict the efficacy of ezetimibe[11]) was investigated (eTable 3 in the Supplement).

For comparison with *NPC1L1*, other LDL-C-lowering alleles in or near genes encoding other current or prospective molecular targets of LDL-C-lowering therapy (ie, *HMGCR, PCSK9, ABCG5/G8, LDLR*) were studied.[11] Three LDL-C-lowering polymorphisms in or near *HMGCR,* previously demonstrated to mimic the efficacy and metabolic effects of statins,[5,11] were analyzed (Table 2). At the *ABCG5/G8* and *LDLR* loci, polymorphisms previously used to investigate genetic relationships between LDL-C and coronary artery disease[11] were studied (Table 2). At the *PCSK9* locus, in addition to the rs11591147 (p.R46L) variant (Table 2), the combined association of up to an additional 8 likely independent LDL-C-lowering polymorphisms was investigated (eFigure 3 in the Supplement). Genetic variants included in the analyses were strongly and specifically associated with LDL-C (eFigure 4 in the Supplement).

Approximate conditional analyses on large-scale LDL-C association data from the Global Lipids Genetics Consortium[18] using the GCTA software[25,26] were performed to identify distinct association signals for LDL-C at the *NPC1L1* and *PCSK9* loci. This approach uses genetic association results in addition to the linkage disequilibrium pattern in a reference population to estimate the association of genetic variants in a region after accounting for 1 or more index genetic variants. In doing so, the software allows for the identification of likely independent association signals in a given region using result-level data. At the *PCSK9* locus, in a smaller sample of individuals with individual-level genotypes, formal conditional analyses of the association with LDL-C of polymorphisms after adjusting for rs11591147 genotype status were conducted (eFigure 3 in the Supplement).

Genetic Reference Information

The HUGO Gene Nomenclature Committee[27] (http://www.genenames.org) gene names of the investigated genes were HGNC:7898 (*NPC1L1*), HGNC:5006 (*HMGCR*), HGNC:20001 (*PCSK9*), HGNC:13886 (*ABCG5*), HGNC:13887 (*ABCG8*), and HGNC:6547 (*LDLR*). Genomic coordinates reported in this article represent the chromosome and physical position of genetic variants according to the Human Reference Genome Build 37 (http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/). Polymorphism names reported in the manuscript represent rsID entries from dbSNP release 147 (http://www.ncbi.nlm.nih.gov/SNP/).

Statistical Analysis

Genetic association data for the meta-analyses were either generated or gathered from available sources at the MRC Epidemiology Unit, University of Cambridge. For each genetic variant and outcome, inverse variance–weighted meta-analysis using fixed-effect models was used to obtain pooled estimates. The I^2 statistic was used to quantify heterogeneity. For each gene, associations of LDL-C-lowering genetic variants and outcomes were estimated using Mendelian randomization statistical methods.[17] Estimates of genetic variant to LDL-C and genetic variant to outcome associations were used to calculate estimates for the LDL-C reduction to outcome association at each gene.[17] When multiple genetic variants at a given gene were included in the model, estimates were pooled with a weighted generalized linear regression method that accounts for the correlation between genetic variants.[17] The correlation values were obtained from the SNAP software[28] or from the 1000 Genomes Project phase 3 data on individuals of European ancestry (http://browser.1000genomes.org/; eTable 4 in the Supplement). Results were scaled to represent the odds ratio (OR) per 1-mmol/L (38.7-mg/dL) for the genetically predicted reduction in LDL-C. Absolute risk differences were estimated assuming that the incidence rate of type 2 diabetes in the EPIC-InterAct study subcohort would be the baseline incidence rate in unexposed individuals (ie, 3.76 incident cases per 1000 person-years of follow-up).[13] This baseline rate was then multiplied by the OR estimated from genetic analyses to obtain the at-risk incidence rate. The absolute risk difference estimate was the at-risk incidence rate minus the baseline incidence rate. Absolute risk differences were expressed in incident events per 1000 person-years for a 1-mmol/L genetically predicted reduction in LDL-C. Statistical analyses were conducted using Stata version 14.1 (StataCorp), R version 3.2.2 (R Foundation for Statistical Computing), and METAL version 2011-03-25.[29] A 2-tailed $P < .05$ was considered statistically significant.

Table 2. Low-Density Lipoprotein Cholesterol–Lowering Polymorphisms at *NPC1L1* and Other Genes and Their Association With Type 2 Diabetes and Coronary Artery Disease

Gene	dbSNP rsID[a]	Genomic Coordinate, Chromosome, and Position[b]	Effect Allele and Other Allele	Effect Allele Frequency, Mean (Range)[c]	No. With LDL-C Level	Change in LDL-C per Allele, β (95% CI), mmol/L[d]	*P* Value	CAD per Allele, OR (95% CI)[e]	*P* Value	Type 2 Diabetes, per Allele OR (95% CI)[f]	*P* Value	I^2, %	*P* Value[g]
NPC1L1	rs2073547	chr7: 44582331	A and G	0.81 (0.81 to 0.82)	169 889	−0.05 (−0.06 to −0.04)	2×10^{-21}	0.98 (0.96 to 1.00)	.09	1.05 (1.03 to 1.08)	2×10^{-5}	0	.48
	rs217386	chr7: 44600695	A and G	0.42 (0.41 to 0.44)	173 021	−0.04 (−0.04 to −0.03)	1×10^{-19}	0.98 (0.96 to 1.00)	.03	1.03 (1.01 to 1.05)	.003	0	.68
HMGCR	rs12916[h]	chr5: 74656539	T and C	0.58 (0.57 to 0.60)	168 357	−0.07 (−0.08 to −0.07)	8×10^{-78}	0.97 (0.95 to 0.98)	2×10^{-4}	1.03 (1.01 to 1.05)	7×10^{-4}	46.4	.03
	rs5744707	chr5: 74890618	A and G	0.90 (0.90 to 0.91)	172 928	−0.06 (−0.07 to −0.04)	6×10^{-19}	0.97 (0.94 to 1.00)	.04	0.98 (0.96 to 1.01)	.24	2.8	.38
	rs16872526	chr5: 74675717	T and G	0.91 (0.90 to 0.92)	173 009	−0.04 (−0.05 to −0.03)	2×10^{-8}	0.99 (0.96 to 1.02)	.44	1.02 (0.99 to 1.05)	.32	50.1	.11
PCSK9	rs11591147	chr1: 55505647	T and G	0.02 (0.01 to 0.02)	77 417	−0.50 (−0.53 to −0.46)	9×10^{-143}	0.77 (0.69 to 0.87)	7×10^{-6}	1.09 (1.01 to 1.17)	.03	0	.39
ABCG5/G8	rs4299376	chr2: 44072576	T and G	0.69 (0.68 to 0.70)	144 861	−0.08 (−0.09 to −0.07)	4×10^{-72}	0.95 (0.93 to 0.97)	1×10^{-6}	1.01 (0.99 to 1.03)	.29	2.2	.38
LDLR	rs6511720	chr19: 11202306	T and G	0.11 (0.10 to 0.12)	170 608	−0.22 (−0.23 to −0.21)	4×10^{-262}	0.88 (0.85 to 0.91)	1×10^{-13}	1.03 (1.00 to 1.06)	.05	0	.93

Abbreviations: CAD, coronary artery disease; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio.

[a] Polymorphism names are rsID entries from dbSNP release 147.

[b] Genomic coordinates represent chromosome and physical position of genetic variants according to the Human Reference Genome Build 37.

[c] Data are from the European Prospective Investigation into Cancer and Nutrition (EPIC)-InterAct study,[13] the UK Biobank study,[14] and the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM).[15]

[d] Data are from the Global Lipids Genetics Consortium.[18]

[e] Data are from the Coronary ARtery DIsease Genome wide Replication and Meta-analysis (CARDIoGRAM) plus the Coronary Artery Disease (C4D) Genetics (CARDIoGRAMplusC4D) Consortium[19] (60 801 cases of CAD and 123 504 controls).

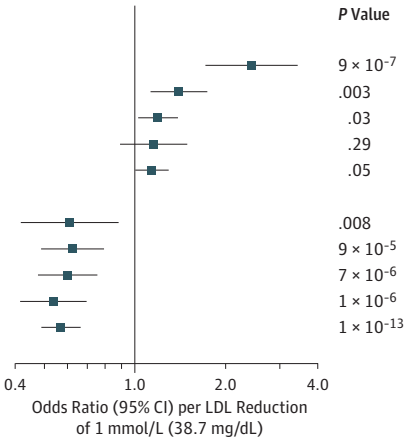
[f] Data are from 50 775 cases with type 2 diabetes and 270 269 controls from the EPIC-InterAct study,[13] the UK Biobank study,[14] and DIAGRAM.[15]

[g] For heterogeneity.

[h] In addition to the EPIC-InterAct study,[13] the UK Biobank study,[14] and DIAGRAM,[15] type 2 diabetes association analyses of rs12916 included 11 studies (4496 cases and 50 677 controls) previously reported by Swerdlow et al.[5] The total sample size of this analysis was based on 55 271 cases with type 2 diabetes and 320 946 controls.

Figure. Association of Low-Density Lipoprotein Cholesterol (LDL-C)–Lowering Genetic Variants With Coronary Artery Disease and Type 2 Diabetes

	No. of Genetic Variants	Odds Ratio (95% CI)	*P* Value
Type 2 diabetes			
NPC1L1	2	2.42 (1.70-3.43)	9×10^{-7}
HMGCR	3	1.39 (1.12-1.73)	.003
PCSK9	1	1.19 (1.02-1.38)	.03
ABCG5/G8	1	1.15 (0.89-1.48)	.29
LDLR	1	1.13 (1.00-1.29)	.05
Coronary artery disease			
NPC1L1	2	0.61 (0.42-0.88)	.008
HMGCR	3	0.62 (0.49-0.79)	9×10^{-5}
PCSK9	1	0.60 (0.48-0.75)	7×10^{-6}
ABCG5/G8	1	0.54 (0.42-0.69)	1×10^{-6}
LDLR	1	0.57 (0.49-0.66)	1×10^{-13}



Coronary artery disease data are from 60 801 cases with coronary artery disease and 123 504 controls from the Coronary ARtery DIsease Genome wide Replication and Meta-analysis (CARDIoGRAM) plus the Coronary Artery Disease (C4D) Genetics (CARDIoGRAMplusC4D) Consortium.[19] Type 2 diabetes data are from 50 775 cases of type 2 diabetes and 270 269 controls from European Prospective Investigation into Cancer and Nutrition (EPIC)-InterAct study,[13] the UK Biobank study,[14] and the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM).[15] In addition to the EPIC-InterAct study,[13] the UK Biobank study,[14] and DIAGRAM,[15] type 2 diabetes association analyses of rs12916 at *HMGCR* included 11 studies (4496 cases and 50 677 controls) previously reported by Swerdlow et al.[5] Therefore, the sample size of *HMGCR* genetic variants association with type 2 diabetes was 55 271 cases of type 2 diabetes and 320 946 controls. All results are scaled to represent the odds ratio per 1-mmol/L (38.7-mg/dL) genetically predicted reduction in LDL-C.

Results

LDL-C-Lowering Alleles at *NPC1L1* and Risk of Type 2 Diabetes

Alleles that lower LDL-C at the *NPC1L1* locus were inversely associated with coronary artery disease (OR for a genetically predicted 1-mmol/L [38.7-mg/dL] reduction in LDL-C of 0.61 [95% CI, 0.42-0.88]; *P* = .008) and directly associated with type 2 diabetes both individually (Table 2) and collectively (OR for a genetically predicted 1-mmol/L reduction in LDL-C of 2.42 [95% CI, 1.70-3.43], *P* < .001; estimated absolute risk difference, 5.3 incident cases per 1000 person-years for a 1-mmol/L genetically predicted reduction in LDL-C; **Figure**). For both polymorphisms, estimates of the association with type 2 diabetes were consistent across the studies included in the meta-analysis (eFigure 1 in the Supplement). In the periphery of the *NPC1L1* locus, approximately 400 kilobases from the lead rs2073547 polymorphism, there was a known association signal for type 2 diabetes and glycemic traits near the *GCK* gene.[15,20,21] After accounting for variation in *GCK*, the association with type 2 diabetes at *NPC1L1* did not change (eTable 3 in the Supplement). Association estimates remained unchanged when modeling the association of 5 polymorphisms previously used by Ference et al[11] as a proxy for NPC1L1 inhibition (eTable 3). In 14 657 cases of type 2 diabetes and 118 854 controls for whom we had access to individual-level genotyping data, there was no evidence of heterogeneity in the association between *NPC1L1* alleles and type 2 diabetes in analyses stratified by age, sex, or BMI (eFigure 5 in the Supplement). In exome-sequencing association results, there was no evidence of enrichment with *NPC1L1* protein truncating alleles in cases with type 2 diabetes compared with controls (OR of 1.12 [95% CI, 0.88-1.43] for type 2 diabetes among individuals carrying a truncating allele [*P* = .34]), but missense variants in *NPC1L1* predicted to be probably deleterious were overrepresented in individuals with type 2 diabetes compared with controls (OR, 1.26 [95% CI, 1.07-1.47]; *P* = .005).

Associations With Type 2 Diabetes at Other Genes

As previously reported,[5,11] LDL-C lowering alleles at *HMGCR* were associated with type 2 diabetes and coronary artery disease in opposite directions (Table 2 and Figure). An association of the loss-of-function p.R46L (rs11591147) variant in *PCSK9* with higher risk of type 2 diabetes was found (OR of 1.19 [95% CI, 1.02-1.38] for type 2 diabetes per 1-mmol/L genetically predicted LDL-C reduction [*P* = .03]; estimated absolute risk difference, 0.7 incident cases per 1000 person-years for a 1-mmol/L genetically predicted reduction in LDL-C). At *PCSK9*, analyses of the LDL-C association data suggested the presence of distinct association signals. In formal conditional analyses, there was evidence of at least 2 distinct association signals (rs11591147 and rs471705; eFigure 3). Using the GCTA software,[25,26] approximate conditional analyses suggested the presence of 9 distinct association signals (rs11591147 plus 8 additional genetic variants; eFigure 3 in the Supplement). Inclusion of these additional signals gave similar associations with type 2 diabetes as the p.R46L variant alone (OR of 1.21 [95% CI, 1.04-1.41] for type 2 diabetes per 1-mmol/L genetically predicted reduction in LDL-C using rs11591147 plus rs471705 [*P* = .01] and OR of 1.16 [95% CI, 1.03-1.31] using rs11591147 plus the 8 additional polymorphisms [*P* = .02]; eTable 3 in the Supplement). The associa-

tion with type 2 diabetes of LDL-C-lowering alleles at the *ABCG5/G8* and *LDLR* loci did not reach statistical significance. There was no evidence of association with type 2 diabetes for missense variants predicted to be probably deleterious or protein truncating alleles in the *HMGCR*, *PCSK9*, *ABCG5, ABCG8* and *LDLR* genes (eTable 5 in the Supplement), but the 95% CIs around the risk estimates were generally wide, reflecting the low prevalence of these genetic variants and the relatively small sample size of this analysis.

Evidence of Gene-Specific Associations With Risk of Type 2 Diabetes

In analyses of the association with disease risk of a given genetically predicted reduction in LDL-C, there was a similar reduction in coronary artery disease risk across genes (I^2 for heterogeneity in genetic associations = 0%, P = .93). However, for the same reduction in LDL-C, the association with type 2 diabetes risk differed by gene (I^2 = 77.2%, P = .002). The different magnitudes and directions of association of LDL-C-lowering alleles with continuous glycemic and anthropometric traits suggested gene-specific mechanisms underlying the altered risk of type 2 diabetes (eFigure 6 in the Supplement). For example, at the *HMGCR* locus there were associations with BMI and waist-to-hip ratio, whereas at the *PCSK9* locus there were associations with higher fasting glucose levels and 2-hour glucose levels (eFigure 6).

Discussion

In this meta-analysis, exposure to LDL-C-lowering genetic variants in or near the *NPC1L1* gene was associated with a higher risk of type 2 diabetes. This finding is consistent with the results of a small-scale open-label RCT showing increased glycated hemoglobin in association with ezetimibe treatment.[30] Blazing et al[31] reported that the addition of ezetimibe to simvastatin for secondary cardiovascular prevention in IMPROVE-IT resulted in a small and not statistically significant increase in risk of new-onset diabetes (9% relative risk increase per 1-mmol/L [38.7-mg/dL] reduction in LDL-C). However, the IMPROVE-IT results may not be sufficient to rule out an effect of inhibiting NPC1L1 on diabetes risk because (1) some of the effects of NPC1L1 inhibition may be apparent only after several years of treatment; (2) the risk of type 2 diabetes in individuals with a history of acute coronary syndrome yet free from type 2 diabetes in IMPROVE-IT may not reflect that of the general population on which this genetic analysis is based; (3) limited compliance to drug treatment, as observed in IMPROVE-IT,[7] may dilute etiological effect estimates. By analogy, the association of statin treatment with higher risk of diabetes was only demonstrable in a meta-analysis of several RCTs including more than 90 000 individuals.[3] Therefore, these results warrant the continued monitoring of the glycemic effects of ezetimibe in RCTs and clinical practice, particularly in a primary prevention setting.

The results of this study show that multiple LDL-C-lowering mechanisms, including those mediated by the molecular targets of available LDL-C-lowering drugs (ie, statins, ezetimibe, and proprotein convertase subtilisin/kexin type 9 [PCSK9] inhibitors), are associated with adverse metabolic consequences and increased type 2 diabetes risk. These findings are consistent with other studies of the association with type 2 diabetes of genetic scores aggregating multiple polymorphisms affecting LDL-C and other lipid fractions.[32] They are consistent with the observation that patients with familial hypercholesterolemia are less likely to have type 2 diabetes.[33] The genes that were associated both with lower LDL-C levels and higher risk of type 2 diabetes have an effect on LDL-C level by distinct pathways including cholesterol absorption (*NPC1L1*),[34] endogenous cholesterol synthesis (*HMGCR*),[35] and internalization of cholesterol-rich particles into the cell (*PCSK9*).[36,37] For a similar reduction in LDL-C, the association with type 2 diabetes differed by gene, which would be consistent with the mediation of their associations by different mechanisms. Besseling et al[33] have proposed that an increased internalization of cholesterol into pancreatic beta cells may result in impaired secretion of insulin, a hypothesis supported by murine experimental models.[38] Alleles that lower LDL-C at *HMGCR* are associated with higher levels of fasting insulin and BMI, suggesting an insulin resistance-related mechanism.[5] In contrast with early evidence showing metabolic benefits of *NPC1L1* knockout in mice,[39] recent studies suggest that its overexpression in the liver may suppress gluconeogenesis and, therefore, that its inhibition could perhaps enhance glucose production.[40] Overall, these results indicate complex relationships between the mechanisms that lead to lower LDL-C and metabolic risk.

Contrary to previous, smaller-scale investigations,[41] there were associations of the p.R46L variant in *PCSK9* (rs11591147) with a higher risk of type 2 diabetes, and higher fasting and 2-hour glucose. These associations have to be interpreted with caution, given the level of statistical significance for the association and the context of multiple comparisons presented in this study. This finding suggests that an effect of LDL-C-lowering drugs on increased risk of diabetes might extend to the newly developed PCSK9 inhibitors, encouraging further genetic and clinical trial investigations.

In general, unlike the association of LDL-C-lowering alleles with cardiovascular risk, the association of these alleles with metabolic risk appears to be specific to particular genes, which in turn might suggest that the adverse consequences of lipid-lowering agents on diabetes risk could be specific to a particular drug target. This may have clinical implications for the future of lipid-lowering therapy in the context of the increasing number of approved drugs acting on different molecular targets. The overall safety profile of these drugs, including the magnitude of risk of new-onset type 2 diabetes, may be relevant to the choice of specific agent for subsets of the patient population (eg, those at high risk for type 2 diabetes who are candidates for lipid-lowering therapy).

A number of assumptions and potential limitations of the genetic approach used in this study should be considered. Mendelian randomization generally assumes that genetic variants are associated with the end point exclusively via the risk factor of interest.[16,17] The strong and specific association with

LDL-C, the well-known role of target genes in LDL-C metabolism and the use of conditionally distinct genetic variants at given loci strengthen the validity of the genetic models used in this study. Similar to previous examples,[5,11,42] the aim of this study was to use genetic variants that mimic the action of pharmacological therapy and therefore pleiotropy (ie, the association with variables other than LDL-C) may be more informative than concerning. For instance, *HMGCR* genetic variants are associated with higher BMI, consistent with the effects on body weight observed in RCTs of statins.[5] However, the consequences of modest reductions in LDL-C associated with LDL-C-lowering alleles over several decades, as assessed in this study, may differ from the short-term pharmacological inhibition of a molecular target in RCTs or clinical practice. In addition, several of the included studies were population-based and therefore association estimates from these studies may not be applicable to patient groups in whom a particular therapy is indicated.

Conclusions

In this meta-analysis, exposure to LDL-C-lowering genetic variants in or near *NPC1L1* and other genes was associated with a higher risk of type 2 diabetes. These data provide insights into potential adverse effects of LDL-C-lowering therapy.

ARTICLE INFORMATION

Author Contributions: Dr Lotta had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Scott and Wareham contributed equally to this article.
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