APOC3 Loss-of-Function Mutations, Remnant Cholesterol, Low-Density Lipoprotein Cholesterol, and Cardiovascular Risk

Mediation- and Meta-Analyses of 137 895 Individuals

Anders B. Wulff, Børge G. Nordestgaard, Anne Tybjærg-Hansen

Objective—Loss-of-function mutations in *APOC3* associate with low remnant cholesterol levels and low risk of ischemic vascular disease (IVD). Because some studies show an additional association with low levels of low-density lipoprotein cholesterol (LDL-C), low LDL-C may explain the low risk of IVD in *APOC3* loss-of-function heterozygotes. We tested to what extent the low risk of IVD in *APOC3* loss-of-function heterozygotes is mediated by low plasma remnant cholesterol and LDL-C.

Approach and Results—In *APOC3* loss-of-function heterozygotes versus noncarriers, we first determined remnant cholesterol and LDL-C levels in meta-analyses of 137 895 individuals. Second, we determined whether the association with LDL-C was masked by lipid-lowering therapy. Finally, using mediation analysis, we determined the fraction of the low risk of IVD and ischemic heart disease mediated by remnant cholesterol and LDL-C. In meta-analyses, remnant cholesterol was 43% lower (95% confidence interval, 40%–47%), and LDL-C was 4% lower (1%–6%) in loss-of-function heterozygotes (n=776) versus noncarriers. In the general population, LDL-C was 3% lower in loss-of-function heterozygotes versus noncarriers, 4% lower when correcting for lipid-lowering therapy, and 3% lower in untreated individuals (*P* values, 0.06–0.008). Remnant cholesterol mediated 37% of the observed 41% lower risk of IVD and 54% of the observed 36% lower risk of ischemic heart disease; corresponding values mediated by LDL-C were 1% and 2%.

Conclusions—The low risk of IVD observed in *APOC3* loss-of-function heterozygotes is mainly mediated by the associated low remnant cholesterol and not by low LDL-C. Furthermore, the contribution of LDL-C to IVD risk was not masked by lipid-lowering therapy. This suggests *APOC3* and remnant cholesterol as important new targets for reducing cardiovascular risk.

Visual Overview—An online visual overview is available for this article. **(*Arterioscler Thromb Vasc Biol*. 2018;38:660-668. DOI: 10.1161/ATVBAHA.117.310473.)**

Key Words: cardiovascular diseases ■ genetics ■ humans ■ risk factors ■ triglycerides

A causal link between *APOC3* loss-of-function mutations and low risk of ischemic vascular disease (IVD) was recently established.[1,2] In both studies, heterozygotes for *APOC3* loss-of-function mutations versus noncarriers had an approximate 40% lower risk of IVD and corresponding 39% to 44% lower plasma levels of triglycerides. Moreover, loss-of-function mutations were also associated with a 46% reduction in levels of apolipoprotein CIII.[2] Similarly, clinical trials with an *APOC3* antisense oligonucleotide (ASO) showed dramatic reductions in levels of triglycerides and very-low–density lipoprotein (VLDL) cholesterol in study participants with a wide range of hypertriglyceridemia[3] or chylomicronemia,[4] but these studies were not designed to evaluate effect on risk of IVD. A biologically plausible explanation for the reduction in risk of IVD observed in the genetic studies mentioned above could, therefore, be the low levels of triglycerides as a marker of low levels of remnant cholesterol, defined as the cholesterol content in the triglyceride-rich lipoproteins, that is intermediate-density lipoproteins and VLDL in the fasting state and these 2 together with chylomicron remnants in the nonfasting state.[5,6] For clinical purposes, remnant cholesterol can, therefore, be easily calculated from a standard lipid profile as total cholesterol minus LDL cholesterol and high-density lipoprotein (HDL) cholesterol[7,8]; a newly developed direct automated measurement of remnant cholesterol is highly correlated with calculated remnant cholesterol.[9] The reason for focusing on remnant cholesterol rather than triglycerides, respectively, the cholesterol and triglyceride content of triglyceride-rich lipoproteins, is that cholesterol and not triglycerides accumulate in the atherosclerotic plaque. However, hydrolysis of triglycerides at the endothelial surface or within the intima may lead to low-grade inflammation and thus explain an inflammatory

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Nonstandard Abbreviations and Acronyms

ASO	antisense oligonucleotide
CETP	cholesterol ester transfer protein
CGPS	Copenhagen General Population Study
CI	confidence interval
HAPI	Heredity and Phenotype Intervention Study
HDL	high-density lipoprotein
IHD	ischemic heart disease
IVD	ischemic vascular disease
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol

component of atherosclerosis.[7,10] Remnant cholesterol has been proposed as a causal factor in the development of IVD because of a strong association between levels of remnant cholesterol and risk of IVD. More recently, Mendelian randomization studies have shown that genetic variants associated with levels of remnant cholesterol also associate with risk of IVD and mortality, directly suggesting causality.[5,6,8,11,12]

However, in the discovery cohorts of some studies, loss-of-function mutations in *APOC3* were also associated with 16% to 17% lower levels of LDL cholesterol.[2,13] Therefore, low LDL cholesterol levels could be responsible for the lower risk of IVD observed in *APOC3* loss-of-function heterozygotes versus noncarriers. It is also possible that the contribution of plasma LDL cholesterol levels to the low risk of IVD in loss-of-function heterozygotes could be masked and underestimated by lipid-lowering therapy: if *APOC3* noncarriers have higher LDL cholesterol levels or more IVD, they might be more likely to receive lipid-lowering therapy, lowering their LDL cholesterol levels and obscuring differences in LDL cholesterol between *APOC3* loss-of-function heterozygotes and noncarriers.

We first determined the levels of remnant cholesterol and LDL cholesterol in *APOC3* loss-of-function heterozygotes versus noncarriers in meta-analyses of 8 study cohorts comprising >137 000 individuals. Second, to determine whether the contribution of LDL cholesterol to IVD risk was masked by lipid-lowering therapy, we compared levels of LDL cholesterol in loss-of-function heterozygotes versus noncarriers in 75 725 individuals from the Danish general population, overall regardless of lipid-lowering therapy, after correction for lipid-lowering therapy, and after excluding individuals on lipid-lowering therapy. Finally, using mediation analysis, we determined the fraction of the observed lower risk of IVD and ischemic heart disease (IHD) associated with *APOC3* loss-of-function mutations, which was mediated by low remnant cholesterol and by low LDL cholesterol, respectively. These questions are clinically important because they indirectly address whether pharmaceutical lowering of triglyceride-rich lipoproteins and remnant cholesterol via *APOC3* inhibition is likely to reduce cardiovascular risk beyond LDL cholesterol lowering.

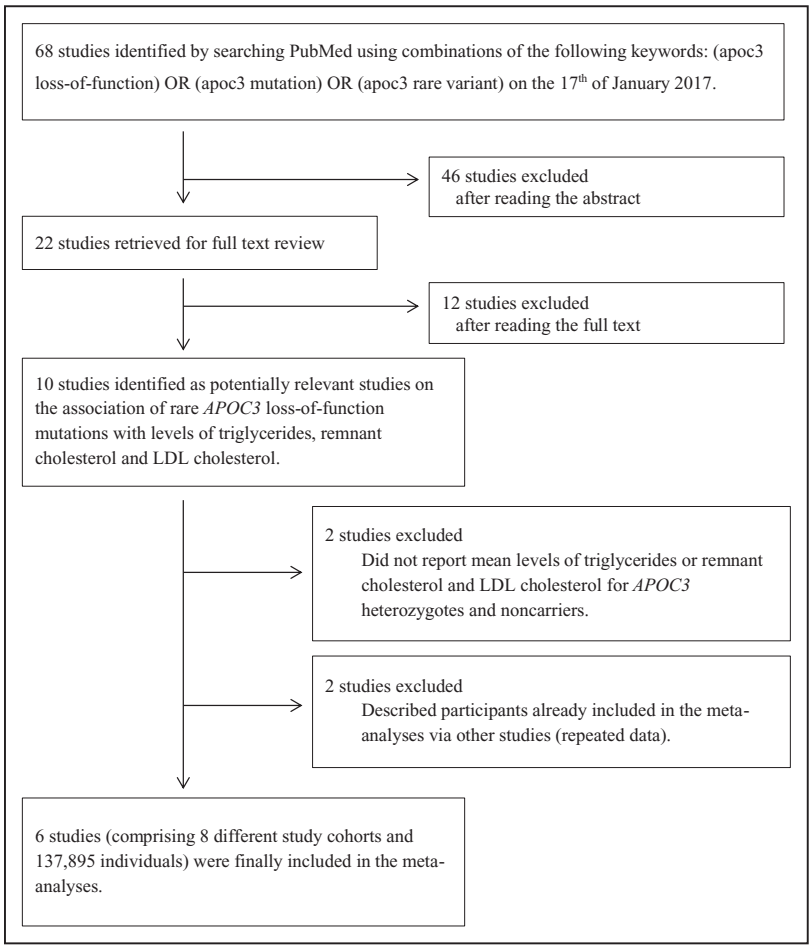


Figure 1. Flowchart for inclusion and exclusion of studies in the meta-analysis. LDL indicates low-density lipoprotein.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Associations and Correlations Between Lipids and Lipoproteins

In the Copenhagen Studies, increased concentrations of plasma triglycerides were associated with increased concentrations of both calculated and measured remnant cholesterol and with reduced concentrations of HDL cholesterol (Figure 1, top). With increasing plasma concentrations of triglycerides, measured remnant cholesterol captured an increasing proportion of calculated remnant cholesterol, from 12% at nonfasting triglycerides <1 mmol/L, ≤21% for triglycerides ≥5 mmol/L. Intercorrelations for all lipids and lipoproteins are shown in Figure 1 (middle). For the 9544 individuals from the CGPS (Copenhagen General Population Study) in which both measured and calculated remnant cholesterol was available,

Table 1. Published Studies of Rare Mutations in *APOC3* and Association With Levels of Remnant Cholesterol and LDL Cholesterol

Reference	Study	Design	Mutation Carrier Status	Allele Frequency, %	No. of Participants	Age, Mean±SD or Median (Interquartile)	Lipid-Lowering Therapy, %	Remnant Cholesterol, mmol/L	Difference, %	*P* Value	LDL Cholesterol, mmol/L	Difference, %	*P* Value
Pollin et al[13]	HAPI	Amish population	Noncarriers		763	44±14	NA	0.29			3.63		
			R19X	2.6	39	42±12	NA	0.16	−46	4×10^{-13}	3.00	−17	0.001
	AFCS excl 335 from the HAPI	Amish population	Noncarriers		656	60±13	NA	0.43			3.63		
			R19X	3.2	42	57±11	NA	0.23	−47	2×10^{-22}	3.29	−9	0.15
Tach-mazidou et al[14]	MANOLIS	Greek island population	Noncarriers		1219	62±20	NA	0.62			3.27		
			R19X	1.9	48	66±17	NA	0.41	−41	1×10^{-11}	3.21	−2	0.77
Jørgensen et al[1]	Copenhagen Studies	General population	Noncarriers		75 465	58 (47–67)	9	0.79			3.32		
			Any mutation	0.2	260	59 (48–69)	6	0.44	−44	2×10^{-54}	3.22	−3	0.06
Crosby et al[2]	Exome sequencing project	Mixed design	Noncarriers		3701	56	NA	0.71			3.79		
			Any mutation	0.4	33		NA	0.43	−39	6×10^{-9}	3.19	−16	0.05
	Replication studies combined	Mixed design	All participants		41 671	55	NA	0.67			3.63		
			Any mutation	0.3	278 (est)		NA	0.41	−39	1×10^{-20}	3.53	−3	0.19
Timpson et al[17]*	UK10K overall	Mixed design	Noncarriers		15 972 (est)†	39	NA	NA			NA		
					15 650 (est)‡								
			c.55+1G>A	0.19	61 (est)†		NA	NA		7×10^{-15}	NA		0.55
					60 (est)‡								
Crawford et al[15]	NHANES meta-analysis	General population	Noncarriers		7591 (est)†	NA	NA	0.70			3.16		
					6492 (est)‡								
			R19X	0.08	12 (est)†		NA	0.31	−51	0.007	3.03	−4	0.68
					10 (est)‡								
Natarajan et al[16]	BioImage	Mixed design	Noncarriers		6331	NA	NA	0.85			3.37		
			Any mutation	0.5	64	NA	NA	0.47	−44	2×10^{-21}	3.41	1	0.75

Remnant cholesterol was calculated as triglycerides divided by 2.2 for all studies. To convert cholesterol from mmol/L to mg/dL, divide by 0.0259. AFCS indicates Amish Family Calcification Study; est, numbers estimated from allele frequency; HAPI, Heredity and Phenotype Intervention Study; LDL, low-density lipoprotein; MANOLIS, Minoan Isolates Study; NA, not available; and NHANES, National Health and Nutrition Examination Surveys.

*This study was not included in meta-analyses because of the lack of availability of data.

†Participants with data on calculated remnant cholesterol.

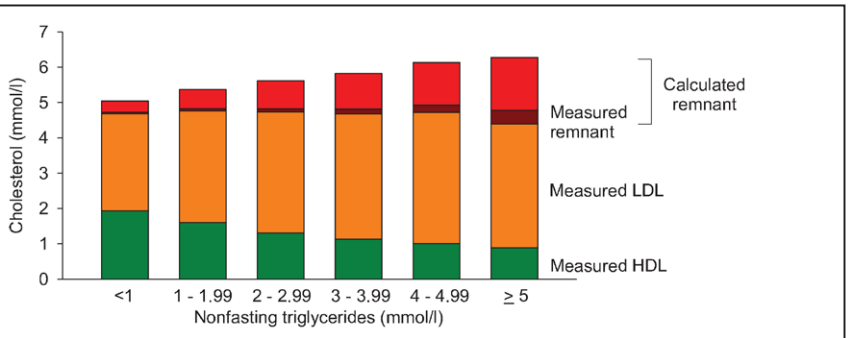
‡Participants with data on LDL cholesterol.

concentrations of measured remnant cholesterol were only 0.14 mmol/L higher per 1 mmol/L increase in calculated remnant cholesterol (corresponding to a 14% increase); however, the 2 types of remnant cholesterol were highly correlated with an R^2=0.51 (Figure 1, bottom; P<0.001).

Remnant Cholesterol and LDL Cholesterol

Eight cohorts from 6 studies were included in the meta-analyses (Table 1; Figures 2 and 3).[1,2,13–16] For remnant cholesterol, these 6 studies included 137 895 individuals of whom 776 were heterozygotes for an *APOC3* loss-of-function mutation, and for LDL cholesterol, they included 136 794 individuals and 774 loss-of-function heterozygotes. *APOC3* loss-of-function heterozygotes had 43% (95% confidence interval [CI], 40%–47%) lower levels of remnant cholesterol compared with noncarriers in both fixed and random effects models. No heterogeneity between studies was observed (I^2=0%; P=0.92; Figure 3, top). For LDL cholesterol, *APOC3* loss-of-function heterozygotes had 4% (95% CI, 1%–6%) lower levels of LDL cholesterol compared with noncarriers in a fixed effects model and 5% (95% CI, 1%–8%) lower levels of LDL cholesterol in a random effects model (Figure 3, bottom). The largest reductions in LDL cholesterol of 16% to 17% were observed in the discovery cohorts of the Pollin and Crosby studies.[2,13] These studies were also the basis for the hypothesis that the observed lower risk in loss-of-function heterozygotes could be explained by the associated reduction in LDL cholesterol. Some heterogeneity between studies was observed (I^2=40%; P=0.11).

In the Copenhagen Studies, 260 heterozygotes for *APOC3* loss-of-function mutations were identified among the 75 725 individuals (Table 2), as described previously.[1] Remnant cholesterol was 44% (0.3 mmol/L) lower (P=1×10^{-51}), and LDL cholesterol was 3% (0.1 mmol/L) lower (P=0.06) in loss-of-function heterozygotes versus noncarriers overall, regardless of lipid-lowering therapy (Figure 4). After correcting for lipid-lowering therapy, loss-of-function heterozygotes had 43% (0.3 mmol/L) lower levels of remnant cholesterol (P=5×10^{-49}) and 4% (0.1 mmol/L) lower levels of LDL cholesterol (P=0.008) compared with noncarriers (Figure 4). Similarly, when excluding participants on lipid-lowering therapy at the time of lipid assessment from the analyses, remnant cholesterol was 44% (0.3 mmol/L) lower (P=2×10^{-49}), and LDL cholesterol was 3% (0.1 mmol/L) lower (P=0.02) in loss-of-function heterozygotes compared with noncarriers (Figure 4). Taken together, reductions in remnant cholesterol and LDL cholesterol levels as a function of *APOC3* genotype were similar in meta-analyses and in the Copenhagen Studies and in the latter were not majorly affected by lipid-lowering therapy.



P value / *R*	Calculated remnant	Measured remnant	LDL cholesterol	HDL cholesterol	Nonfasting triglycerides	Total cholesterol
Calculated remnant		0.72	0.39	-0.58	0.86	0.36
Measured remnant	<0.001		0.24	-0.27	0.83	0.30
LDL cholesterol	<0.001	<0.001		-0.16	0.28	0.91
HDL cholesterol	<0.001	<0.001	<0.001		-0.49	0.18
Nonfasting triglycerides	<0.001	<0.001	<0.001	<0.001		0.27
Total cholesterol	<0.001	<0.001	<0.001	<0.001	<0.001	

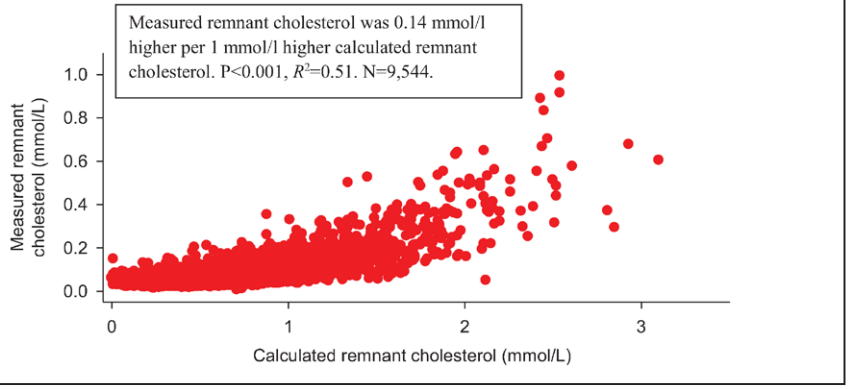


Figure 2. Total plasma lipoprotein cholesterol, calculated remnant cholesterol, measured remnant cholesterol, measured low-density lipoprotein (LDL) cholesterol, and measured high-density lipoprotein (HDL) cholesterol as a function of nonfasting plasma triglycerides (**top**), and pairwise associations between these parameters using Pearson correlation (**middle**). Measured remnant cholesterol as a function of calculated remnant cholesterol (**bottom**). Determined in 9544 participants from the CGPS (Copenhagen General Population Study). *P* values by Pearson correlation (**middle**) and linear regression (**bottom**). To convert mmol/L to mg/dL, divide by 0.0113 for triglycerides and by 0.0259 for cholesterol. *R* indicates correlation coefficient; R^2, coefficient of determination.

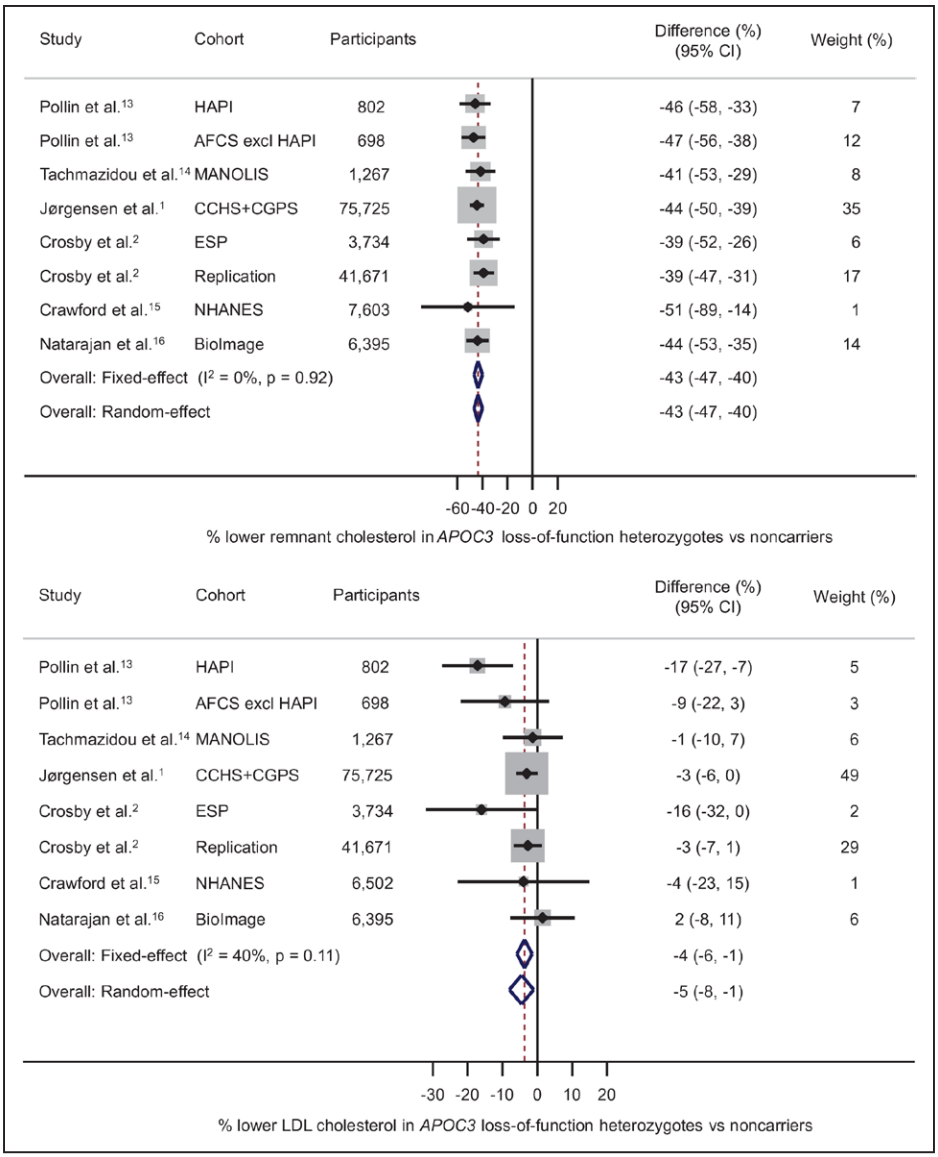


Figure 3. Meta-analyses of difference in levels of remnant cholesterol (**top**) and low-density lipoprotein cholesterol (**bottom**) in *APOC3* loss-of-function heterozygotes vs noncarriers. AFCS indicates Amish Family Calcification Study; CCHS, Copenhagen City Heart Study; CGPS, Copenhagen General Population Study; CI, confidence interval; HAPI, Heredity and Phenotype Intervention Heart Study; I^2, fraction of between study variability because of heterogeneity; MANOLIS, Minoan Isolates Study; NHANES, National Health and Nutrition Examination Surveys; and *P*, heterogeneity assessed by Q statistics.

Other Lipids, Lipoproteins, and Apolipoproteins

Compared with noncarriers, heterozygotes for *APOC3* loss-of-function mutations had median reductions of 47% (0.7 mmol/L) in triglycerides ($P=3\times10^{-54}$), 10% (0.4 mmol/L) in non-HDL cholesterol ($P=2\times10^{-12}$), and 13% (14 mg/dL) in apolipoprotein B ($P=4\times10^{-19}$), and a corresponding increase in levels of HDL cholesterol of 33% (0.5 mmol/L; $P=2\times10^{-29}$; Table 2). There were no differences in baseline characteristics of other risk factors for cardiovascular disease as a function of *APOC3* genotype, suggesting no obvious confounding.

Markers of Liver Function and Inflammation

APOC3 genotype was not associated with plasma levels of high-sensitivity C-reactive protein or alanine aminotransferase, markers of inflammation, and liver damage, respectively (Table 2).

Mediation Analysis

In the Copenhagen Studies, *APOC3* loss-of-function heterozygotes versus noncarriers had a 41% reduction in risk of IVD (hazard ratio, 0.59; 95% CI, 0.41–0.86; $P=0.007$) and a corresponding 36% reduction in risk of IHD (hazard ratio, 0.64; 95% CI, 0.41–0.99; $P=0.04$) as reported previously[1] (Figure 5). The corresponding reductions in remnant cholesterol and LDL cholesterol corrected for lipid-lowering therapy were 43% ($P=5\times10^{-49}$) and 4% ($P=0.008$), respectively (Figures 4 and 5). Using mediation analysis and the product of coefficients method, the lower levels of remnant cholesterol in loss-of-function heterozygotes versus noncarriers mediated 37% of the lower risk of IVD (*P* value for the indirect effect, $P=6\times10^{-37}$) and 54% of the lower risk of IHD (*P* value for the indirect effect, $P=1\times10^{-37}$; Figure 5). In comparison, the 4% lower levels of LDL cholesterol mediated only 1% and 2% of the lower risks

Table 2. Baseline Characteristics by *APOC3* Loss-of-Function Genotype in the Copenhagen Studies

	Noncarriers	Heterozygotes	*P* Value
No. of subjects (%)	75 465 (99.66%)	260 (0.34%)	…
Age, y	58 (47–67)	59 (48–69)	0.09
Women, n (%)	41 765 (55%)	144 (55%)	0.99
Total cholesterol, mmol/L	5.7 (5.0–6.5)	5.7 (4.9–6.3)	0.18
LDL cholesterol, mmol/L	3.3 (2.7–4.0)	3.2 (2.6–3.8)	8E-3
HDL cholesterol, mmol/L	1.5 (1.2–1.9)	2.0 (1.6–2.3)	2E-29
Non-HDL cholesterol, mmol/L	4.1 (3.4–4.9)	3.7 (3.1–4.3)	2E-12
Nonfasting triglycerides, mmol/L	1.5 (1.0–2.2)	0.8 (0.6–1.1)	3E-54
Remnant cholesterol, mmol/L	0.7 (0.5–1.0)	0.4 (0.3–0.5)	5E-49
Apolipoprotein B, mg/dL	106 (87–130)	92 (77–106)	4E-19
Alanine aminotransferase, U/L	19 (14–27)	19 (14–26)	0.30
CRP, mg/L	1.5 (1.1–2.6)	1.7 (1.1–3.1)	0.36
Body mass index, kg/m^2	26 (23–28)	26 (24–28)	0.67
Diabetes mellitus, n (%)	2920 (4%)	13 (5%)	0.35
Smoking, n (%)	17 960 (24%)	67 (26%)	0.46
Hypertension, n (%)	42 552 (56%)	142 (55%)	0.57
Physical inactivity, n (%)	5526 (7%)	25 (10%)	0.16
Alcohol consumption, n (%)	54 317 (72%)	192 (74%)	0.50
Lipid-lowering therapy, n (%)	7046 (9%)	16 (6%)	0.08
Ischemic vascular disease events, n (%)	10 770 (14%)	27 (10%)	0.07
Ischemic heart disease events, n (%)	7537 (10%)	20 (8%)	0.22

Values are median and interquartile range or n. Except for age and body mass index, *P* values are by Mann–Whitney *U* test, and χ^2 test was used for continuous and categorical variables, respectively. For age and body mass index, *P* values are by Student *t* test. Lipid and CRP (C-reactive protein) concentrations are corrected for lipid-lowering therapy. Remnant cholesterol is calculated as total cholesterol−(LDL cholesterol+HDL cholesterol). HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; and n, number of subjects and percentage.

of IVD and IHD, respectively (*P* values for the indirect effect, *P*=0.13 and *P*=0.03; Figure 5). Results were similar when using the difference of coefficients method (data not shown). The full models for the mediation analyses for all lipid parameters are shown for IVD and IHD in Table I in the online-only Data Supplement, including adjustments for C-reactive protein.

Discussion

A principal finding of this study is that heterozygotes for *APOC3* loss-of-function mutations have lifelong 43% lower plasma levels of remnant cholesterol, but only 4% lower levels of LDL cholesterol compared with noncarriers, in studies comprising >137 000 individuals. Furthermore, the lower LDL cholesterol in loss-of-function heterozygotes versus noncarriers was similar in individuals overall, in individuals corrected for lipid-lowering therapy, or when individuals on lipid-lowering therapy were excluded, implying that lipid-lowering therapy is unlikely to mask the contribution of LDL cholesterol to the reduction in risk of IVD. Finally, remnant cholesterol mediated 37% of the observed lower risk of IVD and 54% of the lower risk of IHD in loss-of-function heterozygotes versus noncarriers, whereas LDL cholesterol only mediated 1% and 2% of the lower risk of IVD and IHD, respectively. These results are novel and clinically important because they suggest that the lower risk of IVD associated with loss-of-function mutations in *APOC3*, mimicking *APOC3* antisense therapy, is due mainly to lower levels of remnant cholesterol and not lower LDL cholesterol. This lends support to *APOC3* and remnant cholesterol as important drug targets for reducing cardiovascular risk.

The mechanistic interpretation of the data presented here is likely straightforward: triglyceride-rich remnant lipoproteins, marking remnant cholesterol, can enter and get trapped within the arterial intima.[8,19] Lipoprotein lipase, either at the endothelial surface or within the arterial intima, can then degrade triglycerides leading to the liberation of free fatty acids and monoacylglycerols, both of which are toxic to tissues and thus likely will generate local inflammation.[10,20] Therefore, because *APOC3* loss-of-function mutations cause lifelong low triglycerides and remnant cholesterol, this will lead to less triglyceride-rich lipoproteins and hence less remnant cholesterol entering the intima, less atherosclerosis, and ultimately less IVD and premature death.[5,6,8,10,21]

In a recent short-term phase 2 trial, *APOC3* silencing in hypertriglyceridemic patients using an ASO resulted in robust lowering of plasma triglycerides but dose-dependent increases in LDL cholesterol only in the monotherapy group (in some cases given on top of a statin), whereas no change in LDL cholesterol levels was observed in the *APOC3* ASO add-on to fibrate group.[3] This is in contrast to the results in the genetic studies included in the present analyses and suggests that a more complicated biology may occur to account for what seems to be initially elevated LDL cholesterol in the setting of substantial acute and short-term *APOC3* inhibition, versus the net neutral effects on LDL cholesterol through lifelong genetic *APOC3* loss-of-function. The disparity may reflect an acute increase in the conversion of VLDL to LDL as a result of enhanced lipoprotein lipase activity and subsequent lipolysis, consequent to the sharp decrease in apoC-III (apolipoprotein C-III), remodeling of lipoprotein content by CETP (cholesterol ester transfer protein), and changes in the secretion and catabolism of the LDL particle in patients treated with the ASO as suggested previously,[3] in contrast to the long-term steady state in those with genetic *APOC3* loss-of-function. Other differences include higher mean baseline fasting triglycerides in the treatment arm of the monotherapy group, both compared with the fibrate group in the ASO study (6.7 versus 3.8 mmol/L) and with nonfasting triglyceride levels in the Copenhagen Studies (median, 1.5 mmol/L in noncarriers and 0.8 mmol/L in heterozygotes) and in other genetic studies. In addition, both the monotherapy groups and the fibrate groups included a varying number of individuals

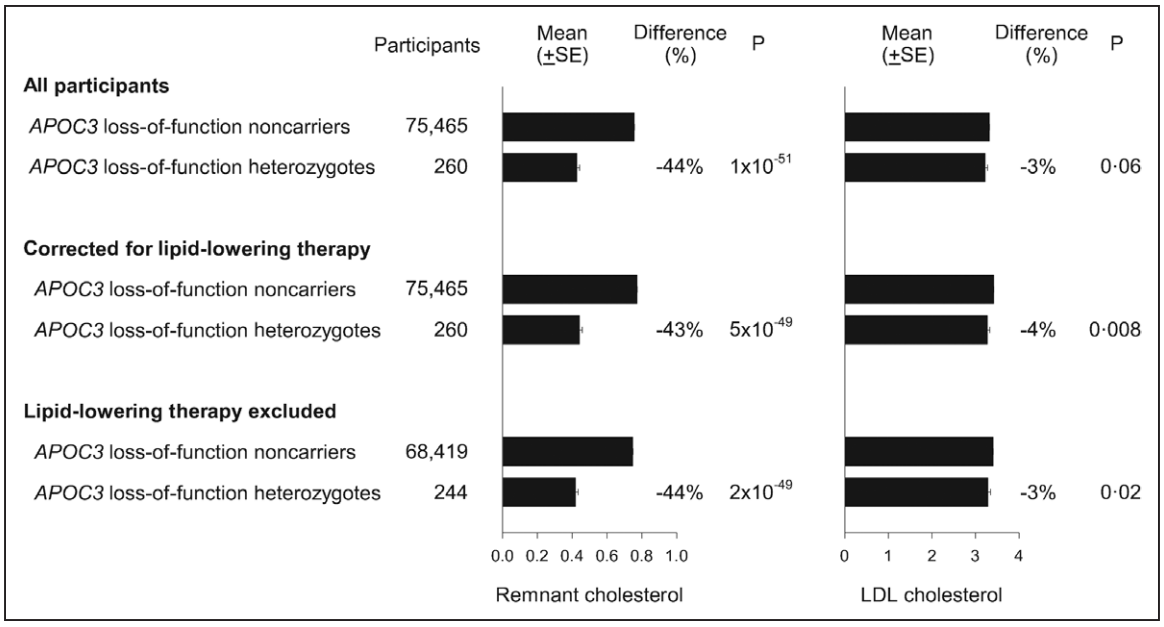


Figure 4. Plasma levels of remnant cholesterol and low-density lipoprotein (LDL) cholesterol in the Copenhagen Studies as a function of *APOC3* genotype. Corrected for lipid-lowering therapy: for participants on lipid-lowering therapy (n=7062), LDL cholesterol was corrected by multiplying by 1.42, and remnant cholesterol was determined as (total cholesterol×1.25)–(LDL cholesterol×1.42)–(high-density lipoprotein cholesterol). For the lipid-lowering therapy–excluded panel, participants on lipid-lowering therapy at the time of lipid assessment were excluded.

with loss-of-function mutations in lipoprotein lipase (from 7% to 39%), in contrast to the populations studied in the present article. Finally, disparities between the studies could be because of unaccounted effects of the ASO or because of genetic *APOC3* loss-of-function. The contribution of these potential mechanisms to the acute, dose-dependent increase in LDL cholesterol remains to be determined.

The present data suggest that the lower risk of IVD observed in *APOC3* loss-of-function heterozygotes is unlikely to be explained by the slightly lower LDL cholesterol levels. This assumption was based on the 16% to 17% reductions in LDL cholesterol observed in the discovery cohorts of the Pollin and Crosby studies (HAPI [Heredity and Phenotype Intervention Study] and ESP [Exome Sequencing Project] in Figure 2),[2,13] whereas the corresponding reductions in the replication cohorts were a nonsignificant 9% and 3%, respectively. Thus, in meta-analysis of 8 studies comparing heterozygotes for *APOC3* loss-of-function mutations with noncarriers, we

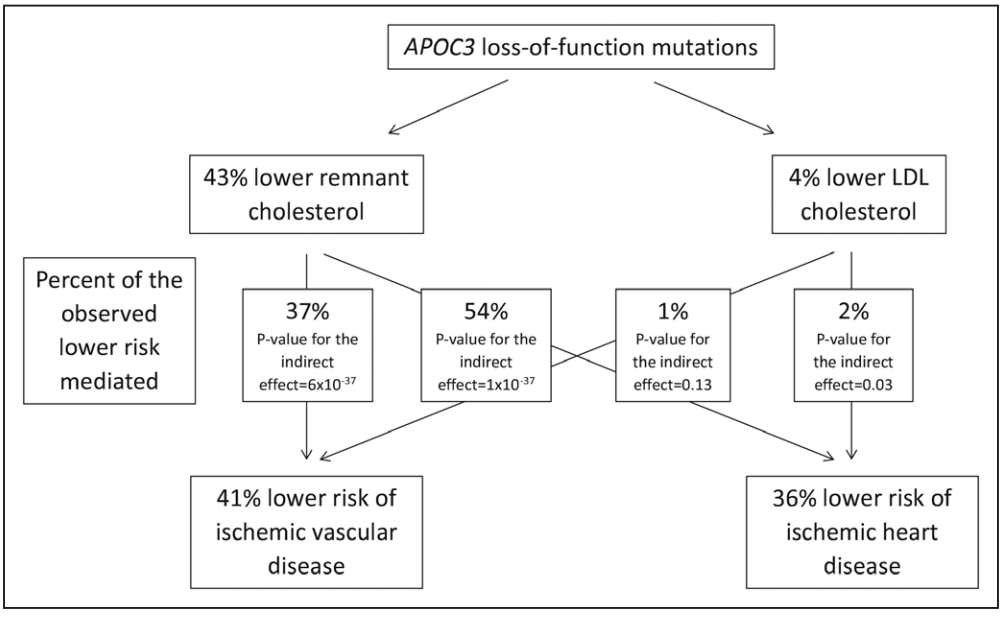


Figure 5. Percentage of the observed 41% lower risk of ischemic vascular disease and 36% lower risk of ischemic heart disease in *APOC3* loss-of-function heterozygotes vs noncarriers[1] mediated by the associated 43% lower remnant cholesterol and 4% lower low-density lipoprotein (LDL) cholesterol observed in the Copenhagen Studies (Figure 3, **middle**). Percentages are calculated as the indirect effect divided by the total effect. The indirect effect and the *P* value for the indirect effect are determined using the product of coefficients method.[18]

observed only a 4% reduction in LDL cholesterol levels (95% CI, 1%–6%). Importantly, not even lifelong genetic reductions in levels of LDL cholesterol of this magnitude can explain the observed ≈40% reduction in risk of IVD. Interestingly, human knockouts, that is, homozygotes for the *APOC3* R19X loss-of-function mutation, identified in a Pakistani cohort with a high rate of consanguinity, did not have low LDL cholesterol levels compared with noncarriers.[22] Finally, the present novel mediation analysis clearly demonstrated that the lower remnant cholesterol in *APOC3* loss-of-function heterozygotes mediated 37% of the lower risk of IVD and 54% of the lower risk of IHD, whereas the lower LDL cholesterol only mediated 1% and 2%, respectively.

In the Copenhagen Studies, with full information on lipid-lowering therapy, the reductions in LDL cholesterol levels observed in *APOC3* loss-of-function heterozygotes versus noncarriers were 3% overall, 4% when correcting for lipid-lowering therapy, and 3% in individuals who were not on lipid-lowering therapy. Thus, lipid-lowering therapy did not mask the contribution of plasma LDL cholesterol levels to the reduction in risk of IVD in *APOC3* loss-of-function heterozygotes.

A novel and important finding in the present study is that reduced remnant cholesterol in *APOC3* loss-of-function heterozygotes mediated 37% and 54% of the reduced risk of IVD and IHD, respectively. However, it is also worth considering whether factors other than plasma levels of remnant cholesterol and LDL cholesterol may contribute to the reduction in IVD risk because *APOC3* genotype also influences plasma levels of apoC-III and HDL cholesterol. High plasma levels of apoC-III is a risk factor for cardiovascular disease. In observational epidemiological studies, blood levels of apoC-III in total plasma or in VLDL and LDL have been associated with cardiovascular events[23–25] leading to the hypothesis that apoC-III might be a causal risk factor for IHD. However, the association between apoC-III and cardiovascular events might also be because of confounding from shared underlying risk factors, such as high plasma triglycerides. Because plasma levels of apoC-III are highly correlated with triglycerides and thus remnant cholesterol,[13,25,26] statistically, this might preclude further discussion on the role of triglycerides and remnant cholesterol versus apoC-III in atherogenesis. Nevertheless, the biologically most likely cause of atherosclerosis is the increase in remnant cholesterol levels because cholesterol and not apoC-III is a major component of the atherosclerotic plaque. ApoC-III has been shown to increase binding to proteoglycans, to increase binding of monocytes to cultured endothelial cells via stimulation of vascular cell adhesion molecule-1 and has emerged as a proinflammatory apolipoprotein in experimental studies, suggesting that apoC-III itself may facilitate atherosclerosis.[27–29]

Although *APOC3* loss-of-function heterozygotes have an approximate 24% increase in levels of HDL cholesterol compared with noncarriers,[1,2,13–15,17] we did not include HDL cholesterol in the mediation analysis. This is because of compelling evidence from both Mendelian randomization studies and clinical trials, which have failed to establish HDL cholesterol as a causal factor in the development of cardiovascular disease.[8,30–32]

Conclusions

In conclusion, the lower risk of IVD observed in heterozygotes for *APOC3* loss-of-function mutations versus noncarriers is largely mediated by the associated substantially lower levels of remnant cholesterol and not by lower levels of LDL cholesterol. This suggests that *APOC3* and remnant cholesterol are important new targets for reducing cardiovascular risk.

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Disclosures

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Highlights

- Heterozygotes for loss-of-function mutations in *APOC3* had 43% lower levels of remnant cholesterol compared with noncarriers but only 4% lower levels of low-density lipoprotein cholesterol.
- Heterozygotes for loss-of-function mutations in *APOC3* had 41% lower risk of ischemic vascular disease compared with noncarriers.
- Low remnant cholesterol mediated 37% of the low risk of ischemic vascular disease in *APOC3* loss-of-function heterozygotes.
- Low low-density lipoprotein cholesterol mediated 1% of the low risk of ischemic vascular disease in *APOC3* loss-of-function heterozygotes.
- An *APOC3* antisense drug, which dramatically decreases plasma levels of triglycerides and remnant cholesterol, is currently under development. Future interventions targeting *APOC3* and remnant cholesterol should be considered for potential clinical application.