Effect of Bile Acid Sequestrants on the Risk of Cardiovascular Events

A Mendelian Randomization Analysis

Stephanie Ross, MSc, PhD; Matthew D'Mello, MSc; Sonia S. Anand, MD, PhD, FRCPC; John Eikelboom, MBBS, MSc; CARDIoGRAMplusC4D Consortium*; Alexandre F.R. Stewart, PhD; Nilesh J. Samani, MD, FRCP; Robert Roberts, MD, FRCPC; Guillaume Paré, MD, MSc;

Background—Statins lower low-density lipoprotein cholesterol (LDL-C) and risk of coronary artery disease (CAD), but they may be ineffective or not tolerated. Bile acid sequestrants (BAS) reduce LDL-C, yet their clinical efficacy on CAD remains controversial.

Methods and Results—We conducted a systematic review and meta-analysis of randomized controlled trials to assess the effect of cholestyramine and colesevelam. We then used Mendelian randomization to estimate the effect of BAS on reducing the risk of CAD. First, we quantified the effect of rs4299376 (*ABCG5/ABCG8*), which affects the intestinal cholesterol absorption pathway targeted by BAS and then we used these estimates to predict the effect of BAS on CAD. Nineteen randomized controlled trials with a total of 7021 study participants were included. Cholestyramine 24 g/d was associated with a reduction in LDL-C of 23.5 mg/dL (95% confidence interval [CI] −26.8,−20.2; N=3806) and a trend toward reduced risk of CAD (odds ratio 0.81, 95% CI 0.70–1.02; P=0.07; N=3806), whereas colesevelam 3.75 g/d was associated with a reduction in LDL-C of 22.7 mg/dL (95% CI −28.3, −17.2; N=759). Based on the findings that rs4299376 was associated with a 2.75 mg/dL decrease in LDL-C and a 5% decrease in risk of CAD outcomes, we estimated that cholestyramine was associated with an odds ratio for CAD of 0.63 (95% CI 0.52–0.77; P=6.3×10^{-6}) and colesevelam with an odds ratio of 0.64 (95% CI 0.52–0.79, P=4.3×10^{-5}), which were not statistically different from BAS clinical trials (P>0.05).

Conclusions—The cholesterol lowering effect of BAS may translate into a clinically relevant reduction in CAD.

(*Circ Cardiovasc Genet*. 2015;8:618-627. DOI: 10.1161/CIRCGENETICS.114.000952.)

Key Words: cholesterol-lowering drugs ■ coronary artery disease ■ genetics ■ lipids ■ Mendelian randomization

Elevated plasma levels of low-density lipoprotein cholesterol (LDL-C) are a well-established risk factor of cardiovascular disease (CVD).[1] Current guidelines recommend that statin therapy should be used in select groups of patients with atherosclerotic CVD in primary and secondary prevention settings.[2] However, statins may not be fully effective in lowering LDL-C[3,4] or well tolerated,[5] and therefore, patients may require additional or alternative lipid-lowering treatments.

Clinical Perspective on p 627

Bile acid sequestrants (BAS) are large polymers that bind to bile salts in the small intestine, preventing their reabsorption into the enterohepatic circulation pathway. The resulting depletion of bile acids leads to increased hepatic metabolism of cholesterol for bile salt synthesis, thereby lowering plasma LDL-C levels.[6] Three BAS have been approved for clinical use: cholestyramine and colestipol (first generation) and colesevelam hydrochloride (colesevelam; second generation). Colesevelam was developed to overcome gastrointestinal intolerance associated with the first-generation BAS.[7–9] Three randomized controlled trials (RCTs) have evaluated the efficacy of cholestyramine for cardiovascular prevention, but results have been inconclusive.[8,10,11] Although most of these trials have demonstrated that treatment with cholestyramine

Received November 28, 2014; accepted May 19, 2015.

From the Population Health Research Institute, Hamilton Health Sciences (S.R., M.D'M., S.S.A., J.E., G.P.), Department of Clinical Epidemiology & Biostatistics, Population Genomics Program (S.R., M.D'M., S.S.A., G.P.), Population Genomics Program, Chanchlani Research Centre (S.R., M.D'M., S.S.A., G.P.), Department of Medicine (S.S.A., J.E.), Department of Pathology & Molecular Medicine (G.P.), Thrombosis & Atherosclerosis Research Institute (G.P.), Hamilton Health Sciences, McMaster University, Hamilton; John and Jennifer Ruddy Canadian Cardiovascular Genetics Centre, University of Ottawa Heart Institute, Ottawa, ON, Canada (A.F.R.S., R.R.); Department of Cardiovascular Sciences, University of Leicester, Leicester, United Kingdom (N.J.S.); National Institute for Health Research Leicester Cardiovascular Biomedical Research Unit, Glenfield Hospital, Leicester, United Kingdom (N.J.S.); and Department of Medicine, University of Ottawa, Ottawa, ON, Canada (R.R.).

*CARDIoGRAMplusC4D Consortium members are included in the Data Supplement.

The Data Supplement is available at http://circgenetics.ahajournals.org/lookup/suppl/doi:10.1161/CIRCGENETICS.114.000952/-/DC1.

Correspondence to Guillaume Paré, MD, MSc, Population Health Research Institute, McMaster University, Hamilton General Hospital Campus, DB-CVSRI, 237 Barton St E, Rm C3103, Hamilton, Ontario, Canada L8L 2X2. E-mail pareg@mcmaster.ca

reduces LDL-C levels, only one trial has shown a modest reduction in the risk of CVD events (odds ratio [OR] 0.81, 95% CI 0.70–1.02; *P*=0.07).[8] To date, there are no adequately powered trials exploring the effects of colesevelam or colestipol on the risk of major cardiovascular events. Thus, the efficacy of BAS in the prevention of CVD is uncertain.

Mendelian randomization analyses use genetic variants with a known biological function to explore the effects of a modifiable exposure on an outcome.[12,13] Genetic variants are useful instruments for assessing causality because they are randomly allocated and they are independent of many factors that may confound observational associations. Thus, in the absence of evidence from randomized trials, the principles of Mendelian randomization can be applied for drug target validation because functional alleles of a gene within a drug target pathway can be used to extrapolate the effects of the pharmacological intervention.[14,15] This approach can strengthen the rationale for conducting an RCT[12] because it is highly cost-effective as a result of the availability of genetic data through large-scale biobanks and data consortia.

The ATP-binding cassette (ABC) genetic subfamily forms active membrane transporters that regulate the delivery and disposal of intestinal cholesterol and affects the same pathway that is targeted by BAS.[16] The ABC subfamily G member 5 (*ABCG5*) and *ABCG8* genes are mainly expressed in hepatocytes and enterocytes.[17] In the liver, these transporter genes are responsible for increased biliary cholesterol secretion, whereas in the intestine, they recycle free cholesterol from the enterocyte back into the intestine lumen and promote the fecal excretion of biliary sterols.[18] The rs4299376 single nucleotide polymorphism (SNP) is an intronic variant of *ABCG8* (Figure I in the Data Supplement). This SNP has been associated with altered plasma LDL-C levels[19–21] and risk of coronary artery disease (CAD) in the CARDIoGRAMplusC4D Consortium.[22] Based on this evidence and the observation that both the ABCG5/8 heterodimer and BAS target intestinal sterol absorption and excretion, the rs4299376 SNP represents a suitable proxy for the mechanism-based effect of BAS on LDL-C and the risk of CVD.

To test whether BAS has the potential to reduce the risk of cardiovascular outcomes, we first conducted a systematic review and meta-analysis to assess the effect of BAS on plasma lipid levels and major cardiovascular outcomes. We then applied principles of Mendelian randomization to predict the effect of BAS on CAD using the known genetic association of the *ABCG5/ABCG8* polymorphism rs4299376 with lipids[23] and CAD.[22]

Methods

Search Strategy and Study Selection of Clinical Trials

A structured search of RCTs evaluating the effects of BAS on markers of cardiovascular risk or clinical outcomes was conducted in the PubMed database. The following terms were used to search all clinical trial registries and databases: colesevelam; cholestyramine; colestipol; placebo; and randomized controlled trials. Only studies with a double-blinded, placebo-controlled trial design in adults aged 18 years that assessed the effect of BAS (ie, cholestyramine, colestipol, and colesevelam) in comparison with a placebo were included. Refer to Methods in the Data Supplement for more details.

Global Lipids Genetics Consortium

Data on the genetic association between the rs4299376 SNP and plasma lipid levels were obtained from a previously published genome-wide association study. In brief, Teslovich et al (2011) performed a meta-analysis of 46 lipid genome-wide association study assessing common variants associated with serum lipids (LDL-C, high density lipoprotein cholesterol (HDL-C), total cholesterol (TC), and triglycerides).[23] A total of 46 studies and 91 285 individuals of European descent were analyzed for the genetic association with LDL-C, whereas data from 95 708, 95 992 and 92 410 individuals were available for HDL-C, TC, and triglycerides, respectively.

CARDIoGRAMplusC4D Consortium

Data on the genetic association between the rs4299376 SNP (*ABCG5/8*) and the risk of CAD was obtained from the CARDIoGRAMplusC4D Consortium. Briefly, the CARDIoGRAMplusC4D Consortium performed a meta-analysis of 63 746 cases of CAD and 130 681 controls.[22] CAD outcomes were defined as one of the following: myocardial infarction (MI), >50% stenosis in at least one coronary vessel at angiography, history of percutaneous transluminal coronary angioplasty or coronary artery bypass graft surgery, angina, or death caused by CAD.[24] For the association between the rs4299376 SNP and CAD outcomes, the lipid-lowering allele was used as reference throughout the article.

Cholesterol Treatment Trialists' Collaboration

As a sensitivity analysis, we confirmed the predicted effect of BAS on CAD using data from the Cholesterol Treatment Trialists' (CTT) Collaboration.[25] Briefly, the CTT was a prospective meta-analysis of 169 138 individuals from 26 statin RCTs that assessed the association between the change in LDL-C with statin therapy and the reduction in risk of CVD. Over a period of 5 years, there were a total of 24 323 major vascular events, which was defined as the first occurrence of coronary death or nonfatal MI, coronary revascularization, or stroke.

Statistical Analysis

To calculate the effect of BAS on plasma lipids levels, the mean change-from-baseline of plasma lipids in the 24 g/d cholestyramine treatment group and the 3.75 g/d colesevelam group were compared with the mean differences in the placebo group. Meta-analyses were performed using an inverse variance random effect meta-analysis. Unless otherwise specified, a correlation coefficient (r) of 0.5 for the difference in the mean change from baseline was assumed for all analyses. Thus, the r was varied by 0.3 and 0.7 for all the relevant studies to determine whether this altered the reported estimates (Figures II–V in the Data Supplement). Refer to Methods in the Data Supplement for further details.

Simulations were performed to predict the effect of 24 g/d cholestyramine on plasma lipid profiles (HDL-C, TC, and triglycerides) using the known genetic associations of rs4299376 SNP with lipids fractions. To do so, we adapted the method from Sofat et al[14] to match the genetic effects to the effect of cholestyramine 24 g/d on LDL-C, taking into account the uncertainty of both the genetic and drug effect estimates. Refer to Methods in the Data Supplement for more information. To validate whether the rs4299376 SNP had a similar effect on plasma lipid profiles as cholestyramine, the predicted effects of cholestyramine on plasma levels of HDL-C, TC, and triglycerides were estimated using genetic data. These predicted estimates were then compared with known effects of cholestyramine on the same lipids fractions from clinical data. Next, the predicted effect of cholestyramine on the risk of cardiovascular outcomes was projected using data from the genetic association of rs4299376 with CAD. This was then compared with the effect of cholestyramine on CAD from the only outcome trial of cholestyramine, Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT).[8] Figure 1 represents the schematic representation of the Mendelian randomization design. As a sensitivity analysis, the predicted effect of cholestyramine on CAD was also estimated using data from the CTT,[25] a

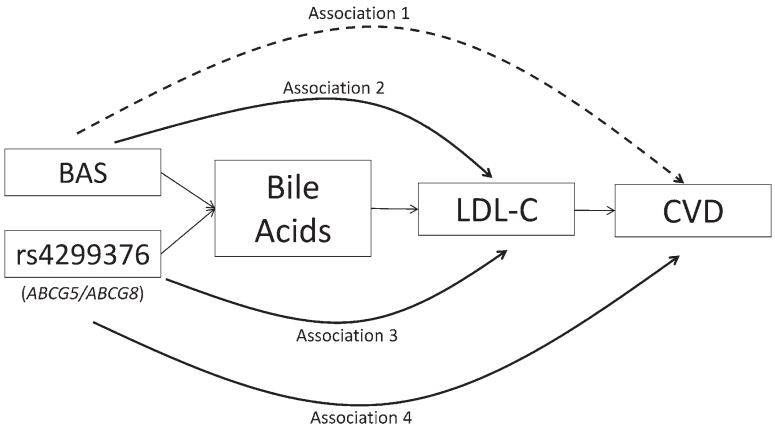


Figure 1. Schematic representation of the Mendelian randomization design. Association 1 represents the effect of bile acid sequestrants (BAS) on the risk of coronary artery disease (CAD). This association was directly obtained from randomized controlled trials (RCTs) that assessed the effect of BAS compared with a placebo and estimated through Mendelian randomization analysis using Associations 2, 3, and 4. Association 2 represents the effect of BAS (ie, 24 g/d cholestyramine or 3.75g/d colesevelam) on the mean change in low-density lipoprotein cholesterol (LDL-C), and data for this association was obtained from RCTs that assessed the effect of BAS compared with a placebo. Association 3 represents the genetic effect of rs4299376 on change in LDL-C, and data for this association was obtained from the Global Lipids Genetics Consortium. Association 4 represents the genetic effect of rs4299376 on the risk of CAD, and data for this association was obtained from the CARDIoGRAMplusC4D Consortium. CVD indicates cardiovascular disease.

large meta-analysis that assessed the effect of statin therapy on the risk of CVD outcomes among 5 trials that compared more intensive to less intensive statin therapy (N=39 612) and 21 trials that compared statin to a control (N=129 526). This estimate was similarly compared with the effect of BAS on cardiovascular outcomes reported in the LRCCPPT, thus testing whether the effect of BAS on reduction of CAD event was consistent with the one observed with statins, after taking into account the differences in LDL-C lowering efficacy. The same analyses were performed for 3.75 g/d colesevelam. Refer to Methods in the Data Supplement for more information. All statistical analyses were performed using R.

Results

Study Selection

The structured literature search of PubMed databases derived a total of 420 citations, and 19 studies were identified for inclusion in this review. Figure VI in the Data Supplement contains a flow diagram of the study selection process. Owing to the lack of reported data from clinical trials, the results of the colestipol meta-analysis are described in Methods in the Data Supplemental and Table I in the Data Supplement.

Randomized Controlled Trials of Cholestyramine

We identified a total of 6 RCTs comprising 4598 hyperlipidemia participants.[8,10,11,26–28] The mean age of these study participants was 48.2 years, whereas 4.8% were female and 95% were European (Table). In the pooled analysis of plasma lipid levels, 3 RCTs evaluated the effect of 24 g of cholestyramine daily dose compared with matching placebo in 4002 hyperlipidemia patients (Figure 2). The pooled estimates indicate that cholestyramine treatment resulted in a mean decrease of LDL-C by 53.4 mg/dL (95% CI −91.8, −15.0) and a decrease of TC by 50.7 mg/dL (95% CI −89.9, −11.5). There was significant heterogeneity among the pooled changes in LDL-C (I^2 93.3% and P for heterogeneity, 5.4×10^{-6}) and TC (I^2 93.5% and P for heterogeneity, 9.1×10^{-6}). Two pooled studies (196 participants) demonstrated a nonsignificant effect in the change of HDL-C and triglycerides (2.6 mg/dL [95% CI −1.2, 6.5] and 3.1 mg/dL [95% CI: −15.5, 21.7], respectively). One study (80 participants) reported a significant decrease of apoB by 44.0 mg/dL (95% CI −61.7, −26.3) and a nonsignificant effect in the change of apoA (10.0 mg/dL [95% CI −3.9, 23.9]). One RCT reported the effect of cholestyramine (24 g/d) on cardiovascular outcomes,[8] randomizing 3806 patients, 342 of whom experienced an event. Cholestyramine did not significantly reduce the composite of cardiovascular death or myocardial infarction (OR 0.81, 95% CI 0.65–1.02, P=0.07), cardiovascular mortality (OR 0.78, 95% CI 0.48–1.27, P=0.322), or myocardial infarction (OR 0.81, 95% CI 0.63–1.03, P=0.082).

Randomized Controlled Trials of Colesevelam

We identified 10 trials with a total of 1142 participants with hyperlipidemia and 883 participants with type 2 diabetes mellitus.[20,29–37] Among all of these participants, the average age was 55.5 years, 51% were women, and 62% were European (Table). Seven RCTs comprising 767 study participants evaluating the effect of colesevelam 3.75 g daily compared with matching placebo were used in the primary analysis (Figure 3). Treatment with colesevelam resulted in a mean decrease of LDL-C by 22.7 mg/dL (95% CI −28.3, −17.2) with significant heterogeneity among the pooled change in LDL-C (I^2 56.95% and P for heterogeneity, 0.032). Colesevelam treatment was also associated with a decrease in TC by 19.2 mg/dL (95% CI −24.4, −14.0), whereas the effect was attenuated in HDL-C and triglycerides (0.30 mg/dL [95% CI −0.14, 2.0] and 9.8 mg/dL [95% CI −1.8, 21.4], respectively). Five pooled studies (628 participants) demonstrated a decrease of apoB by 14.0 mg/dL (95% CI −17.7, −10.3) and had a nonsignificant effect in the change of apoA (1.8 mg/dL [95% CI −0.8, 4.5]). We were unable to conduct subgroup analyses to explore the presence of heterogeneity among pooled estimates owing to a lack of data.

Table. Studies Contributing to the BAS Meta-Analysis

Author and Date	Patient Population	Total Sample Size	Intervention	Comparison	Follow-Up	Age (Mean, SD)	Women, %	European, %	LDL-C, mg/dL*	
									Baseline	End Point
Cholestyramine										
Betteridge 1992[26]	Hyperlipidemia	128	Pravastatin (20 mg bid); Cholestyramine (16–24 g/d)	Placebo	12 weeks	18–70	36 (28)	NR	295 (8.9)	203.8 (NR)
LRCCPPT 1984[8]	Hyperlipidemia	3806	Cholestyramine (24 g/d)	Placebo	7.4 y	47.8	0 (0)	3635 (95.5)	215.6 (NR)	174.9 (NR)
NHLBI Type II Coronary Intervention Study 1984[11]	Hyperlipidemia	143	Cholestyramine (24 g/d)	Placebo	5 y	46.3 (0.55)	28 (20)	135 (94)	241.8 (6.5)	237.4 (6.2)
Pravastatin Multicenter Study Group II 1993[27]	Hyperlipidemia	311	Pravastatin (20 mg/bid); Pravastatin (40 mg/bid); Cholestyramine (12 g/bid); Pravastatin (20 mg bid); and Cholestyramine (12 g bid)	Placebo	8 weeks	51.9	95 (31)	298 (96)	236 (6.6)	162 (6.6)
Watts 1992[10]	Hyperlipidemia	90	Diet and cholestyramine (8 g/d); Diet	Placebo	3.5 months	50.8 (4.7)	0 (0)	NR	203.4 (8.5)	130.3 (7.4)
Wiklund 1990[28]	Hyperlipidemia	120	Pravastatin (10–20 mg/bid); Cholestyramine (24 g/d to highest dose)	Placebo	12 weeks	50.6 (13)	60 (50)	NR	304.6 (68.0)	214.6 (68.0)
Colesevelam										
Bays 2008[29]	Diabetes mellitus	316	Colesevelam (3.75 g/d) with DM drugs	Placebo with DM drugs	26 weeks	56.3 (9.6)	152 (48)	183 (58)	105.6 (33.8)	91.7 (39.1)
Davidson 1999[30]	Hyperlipidemia	147	Colesevelam (1.5 g/d; 2.25 g/d; 3.0 g/d; or 3.75 g/d)	Placebo	6 weeks	56.0 (11)	82 (56)	121 (82.3)	202 (26)	163 (27)
Davidson 2001[20]	Hyperlipidemia	135	Colesevelam (2.3 g/d); Lovastatin (10 mg/d); Colesevelam (2.3 g/d); and Lovastatin (10 mg/d)	Placebo	4 weeks	57.8 (13.4)	72 (53)	112 (83)	172 (5)	158 (5)
Devaraj 2006[31]	Hyperlipidemia	48	Colesevelam (3.75 g/d)	Placebo	6 weeks	NR	NR	NR	150 (33)	136 (37)
Handelsman 2010[32]	Diabetes mellitus	216	Colesevelam (3.75 g/d)	Placebo	16 weeks	54.5 (11.7)	149 (69)	25 (12)	132.8 (23.9)	114.3 (NR)
Hunninghake 2001[33]	Hyperlipidemia	94	Colesevelam (3.8 g/d); Atorvastatin (10 mg/d); Colesevelam (3.8 g/d); and Atorvastatin (10 mg/d); or Atorvastatin (80 mg/d)	Placebo	4 weeks	57.2 (11.4)	37 (39)	NR	184 (5)	163 (8)
Insull 2001[34]	Hyperlipidemia	467	Colesevelam (2.3 g/d; 3.0 g/d; 3.8 g/d; or 4.5 g/d)	Placebo	24 weeks	56 (12)	235 (50)	419 (90)	155 (17)	127 (23)
Knapp 2001[35]	Hyperlipidemia	251	Colesevelam (3.8 g/d); Simvastatin (10 mg/d); Colesevelam (3.8 g/d) and Simvastatin (10 mg/d); Colesevelam (2.3 g/d); Simvastatin (20 mg/d); or Colesevelam (2.3 g/d) and Simvastatin (20 mg/d)	Placebo	6 weeks	54.7 (12.4)	118 (47)	237 (94.4)	198 (39)	167 (46)
Rosenstock 2010[36]	Diabetes mellitus	286	Colesevelam (3.75 g/d) with DM drugs	Placebo with DM drugs	16 weeks	53.3 (10.8)	161 (56.3)	41 (14)	130 (NR)	120.2 (NR)
Zieve 2007[37]	Diabetes mellitus	65	Colesevelam (3.75 g/d)	Placebo	12 weeks	56.2 (9.3)	29 (44.6)	35 (53.8)	122.6 (32.7)	107.8 (27.5)

LRCCPPT indicates Lipid Research Clinics Coronary Primary Prevention Trial; and NR, not reported.
*Refers to the highest single BAS dose reported in the study.

Predicted Effects of BAS on Plasma Lipids Using Genetic Data

Teslovich et al (2010) confirmed the association between the rs4299376 SNP and plasma lipid levels.[23] The rs4299376 polymorphism was significantly associated with a decrease in LDL-C of 2.75 mg/dL per allele (95% CI −3.14, −2.36; $P=1.73\times10^{-47}$), a decrease in TC of 3.01 (95% CI −3.44, −2.58) mg/dL per allele ($P=4.0\times10^{-45}$), a decrease in

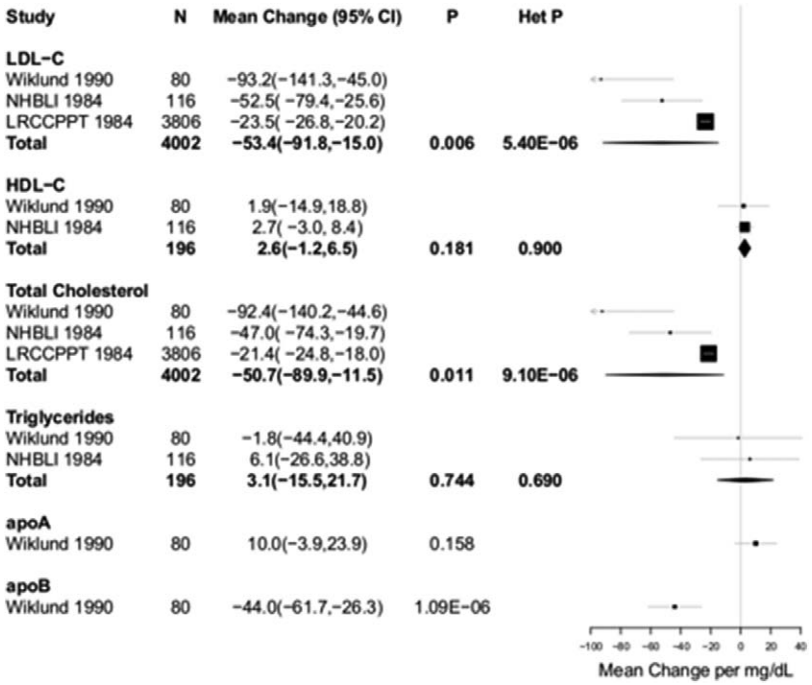


Figure 2. Forest plot of the association of 24 g/d of cholestyramine treatment and the mean difference of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol, triglycerides, apolipoprotein (apo)A and apoB. Het P refers to the heterogeneity *P* value.

triglycerides of 1.08 (95% CI −1.80, −0.36) mg/dL per allele (*P*=0.003), and had a null effect on HDL-C levels (0.05 mg/dL per allele, 95% CI −0.09, 0.19; *P*=0.212). We also explored whether the rs4299376 SNP had potential pleiotropic effects on the risk of diabetes mellitus or on the change in glycohemoglobin (HbA_{1c}), fasting glucose, systolic blood pressure, diastolic blood pressure, and body mass index using data from the DIAGRAM,[38] MAGIC,[39,40] GIANT,[41] and ICBP[42] consortia. We did not observe any significant changes among these traits (*P*>0.05 for all; Figure 4; Table II in the Data Supplement). Next, we sought to determine whether the predicted effect of BAS using genetic data had a similar effect on plasma lipids levels as compared with the reported pharmacological effect. To do so, we adjusted the per-allele genetic effect to match the LDL-C reducing effect of 24 g/d cholestyramine, as reported in the LRCCPPT trial[8] (the only BAS outcome trial available). We then predicted the effect of cholestyramine on TC using genetic data and compared it to the known effect of cholestyramine. The predicted reduction of TC was 25.8 mg/dL (95% CI −32.3, −19.4), which was not statistically different from the reported trial estimate (*P* for difference >0.05).

We performed a similar analysis using the effect of colesevelam 3.75 g/d on LDL-C as the reference for the genetic effect (Figure 5). The predicted reduction of TC by colesevelam was estimated at 25.0 mg/dL (95% CI −33.0, −16.9), which was not different (*P*>0.05) from results of our meta-analysis. The predicted effect on HDL was null (0.42 mg/dL, 95% CI −0.78, 1.61) and was consistent with the reported effect of colesevelam (*P* for difference >0.05). The predicted effect of colesevelam was associated with a modest decrease in triglycerides (8.94 mg/dL [95% CI: −15.5, −2.32] and was statistically different from the observed drug effect (*P* for difference, 0.001).

Predicted Effects of BAS on Cardiovascular Outcomes Using Genetic Data

Data from the CARDIoGRAMplusC4D Consortium was obtained to assess the association of rs4299376 with risk of CAD. The minor allele (LDL-C decreasing) of rs4299376 was associated with a modest yet significant decrease in risk of CAD (OR 0.95, 95% CI 0.93–0.97; $P=2.85\times10^{-7}$). We then derived the predicted effect of 24 g/d cholestyramine on risk of CAD based on the association of the *ABCG5/8* rs4299376 polymorphism on CAD, adjusting the per-allele genetic effect to match the LDL-C reducing effect of 24 g/d cholestyramine. Cholestyramine 24 g/d was predicted to significantly reduce the risk of CAD (OR 0.63, 95% CI 0.52–0.77; $P=6.3\times10^{-6}$). The predicted estimate was not significantly different from the effect observed in the only outcome trial of cholestyramine, LRCCPPT (*P* for difference >0.05; Figure 6). The effect of rs4299376 was also matched to the LDL-C reducing effect of 3.75 g/d colesevelam, leading to a predicted CAD reduction of OR=0.64 (95% CI 0.52–0.79; $P=4.3\times10^{-5}$) with colesevelam 3.75 g/d (*P* for difference >0.05; Figure 6).

Predicted Effect of BAS on Cardiovascular Outcomes Based on CTT Data

As a sensitivity analysis, we used estimates from the CTT to determine whether the effect of BAS on reduction of CAD event was consistent with the one observed with statins by matching the LDL-C lowering effect from LRCPPT to the reported effect from CTT, a large meta-analysis evaluating the effect of cholesterol reduction on CVD.[25] The change in LDL-C levels from 24 g/d cholestyramine was predicted to significantly decrease the risk of major vascular events (OR 0.86, 95% CI 0.85–0.87; $P=6.6\times10^{-83}$; Figure 6). This estimate was not significantly different from observed effect of cholestyramine from clinical trial[8] (LRCCPPT; *P* for difference >0.05). Similarly, the effect of 3.75 g/d colesevelam was also predicted

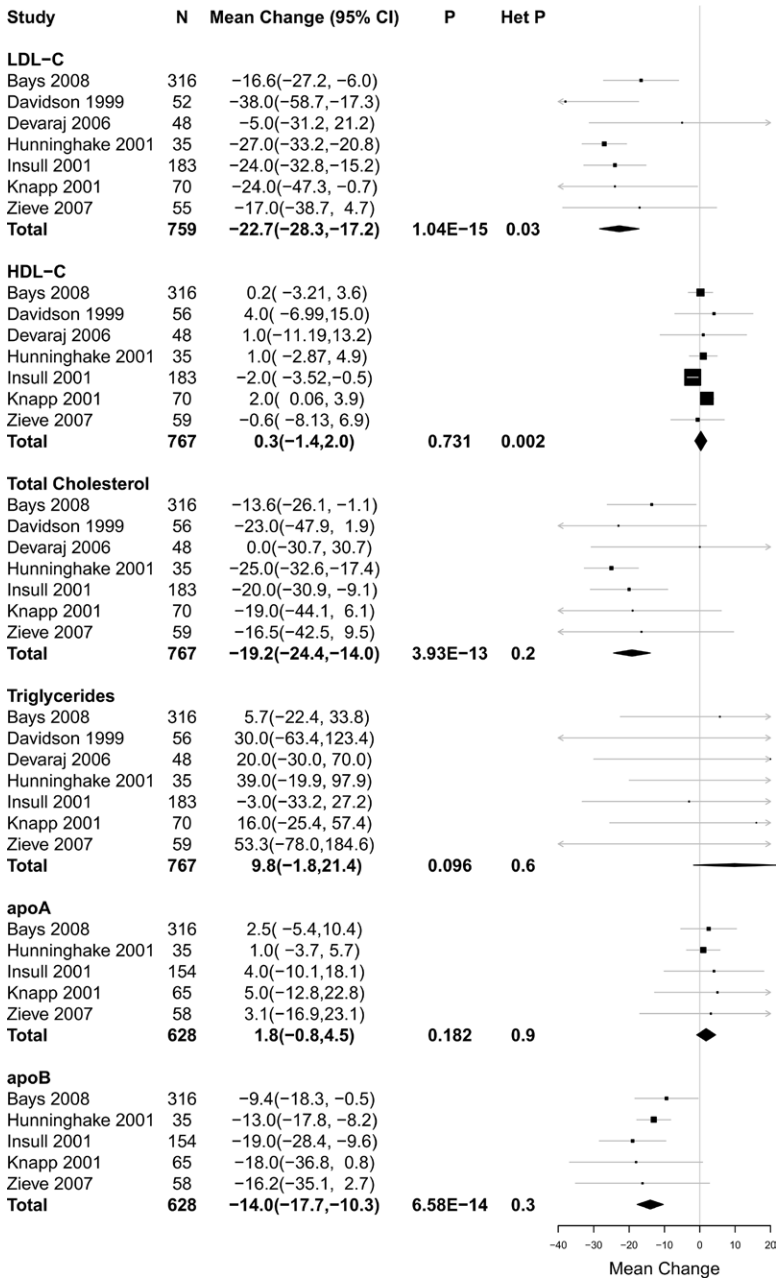


Figure 3. Forest plot of the association of 3.75 g/d of colesevelam treatment and the mean difference of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol, triglycerides, apolipoprotein (apo)A and apoB. Het P refers to the heterogeneity *P* value.

to significantly reduce the risk of cardiovascular events (OR 0.90, 95% CI 0.87–0.93; $P=1.3\times10^{-13}$; *P* for difference >0.05).

Discussion

Mendelian Randomization analyses use the random allocation of alleles to replicate the randomization process in double-blinded clinical trials and to reduce the potential effects of reverse causation and confounding factors. The results of our Mendelian randomization analysis suggest that BAS may be effective in the prevention of CAD. Thus, when given in currently recommended doses, our data demonstrates that cholestyramine and colesevelam were associated with a reduced risk of CAD. Furthermore, our projections concerning the effect of BAS on clinical outcomes were consistent with estimates obtained from the cholestyramine LRCCPPT trial and the CTT.

The predicted effects of BAS on cardiovascular outcomes were based on robust genetic data, which was collectively derived from 194 427 participants from the CARDIoGRAMplusC4D Consortium and 95 708 participants from the Global Lipids Genetics Consortia, respectively. Leveraging already available genetic data is highly cost-effective and has the added advantage of providing estimates that reflect lifelong difference in plasma LDL-C levels between carriers and noncarriers of the rs4299376 allele. In contrast, randomized trials are complex, expensive, and are generally restricted to several years of follow-up, which limits the ability to assess the long-term effects of BAS on clinical outcomes.

Our findings have important clinical implications. Although BAS monotherapy may not be as effective as statin therapy, our results suggest that BAS are likely to be an effective second-line therapy. In contrast, adequately powered randomized

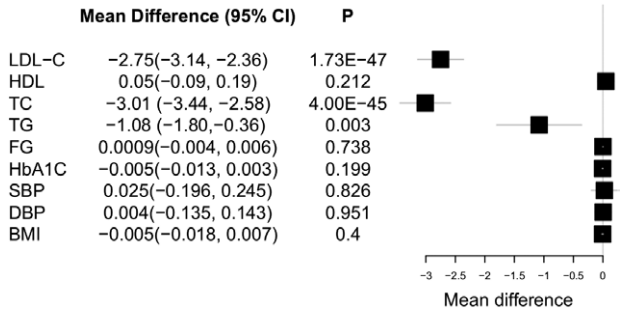


Figure 4. Association of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglycerides (TG), glycohemoglobin (HbA1c), fasting glucose (FG), systolic blood pressure (SBP), diastolic blood pressure (DBP), and body mass index (BMI) among rs4299376 carriers.

trials have failed to show a benefit of Niacin and CETP inhibitors.[43–45] There has also been a shift in clinical guidelines, where patients are more likely to be prescribed with high dose statin therapy to reduce the risk of CAD irrespective of meeting specific LDL-C targets.[2] However, statin therapy may not be well-tolerated or effective in all patients, and the addition of BAS in combination with statin therapy may further prevent the risk of CAD. Even though there is clinical evidence demonstrating that cholestyramine effectively reduces LDL-C levels, as well as suggestive evidence that it decreases the risk of CAD events, its use is hampered by poor patient tolerability and adverse side effects.[6] Colesevelam is much better tolerated,[46,47] has other potential benefits, such as reducing fasting blood glucose levels,[48] and in our Mendelian randomization analysis produced a similar reduction in CAD to that of cholestyramine. Furthermore, our results were also supported by studies that assessed the effect of the cholesterol-lowering agent ezetimibe on CVD risk using both clinical and genetic data. For instance, the IMPROVE-IT trial demonstrated that the addition of ezetimibe to statin therapy resulted in an additional reduction in CVD risk as compared with statin therapy alone.[49] Additionally, genetic studies have also showed that mutations known to inactivate NPC1L1 were associated with lower levels of plasma LDL-C and a reduced risk of CAD.[50] Thus, our results suggest a beneficial effect of colesevelam on risk of CAD and highlight the need for well-designed RCTs

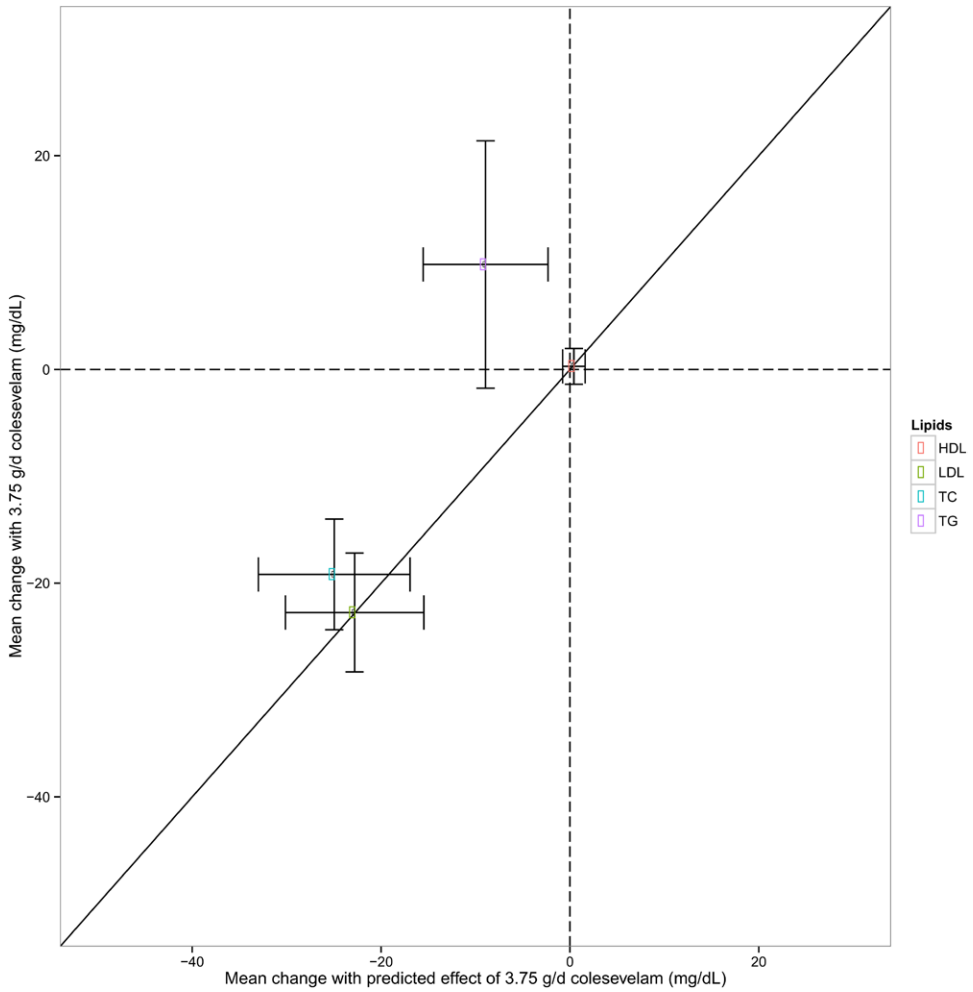


Figure 5 Predicted effects of 3.75 g/d colesevelam on low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), and triglycerides (TG) compared with corresponding pharmacological effect of 3.75g/d colesevelam. This figure illustrates the comparison of the predicted effect of 3.75 g/d colesevelam using genetic data with the effect of 3.75 g/d colesevelam using clinical data. Each point estimate with 95% confidence interval represent the mean change of plasma lipid levels for both the predicted effect of 3.75 g/d colesevelam using genetic data and the effect of 3.75 g/d colesevelam.

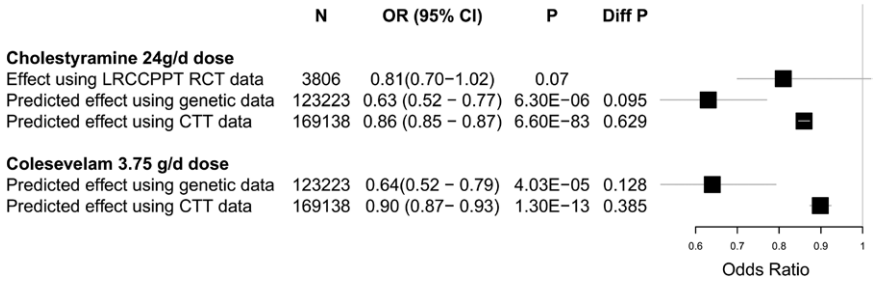


Figure 6. Comparison of the effects of bile acid sequestrants (BAS) on coronary artery disease (CAD) outcomes from the Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT) clinical trial to predicted effects using genetic data. This figure represents the point estimates with 95% confidence intervals of the effect of 24 g/d cholestyramine on CAD using clinical data from the LRCCPPT Trial, the predicted effect of 24 g/d cholestyramine on CAD using genetic data, and the predicted effect of 24g/d cholestyramine on CAD using data from the Cholesterol Treatment Trialists' (CTT). It also shows the point estimates with 95% confidence intervals of predicted effect of 3.75 g/d colesevelam on CAD using genetic data and the predicted effect of 3.75 g/d colesevelam on CAD using data from the CTT. The predicted effect of colesevelam using genetic data and CTT were compared with the LRCCPPT Trial because no outcome trial data were available for colesevelam. The predicted effect of 24 g/d cholestyramine and 3.75 g/d colesevelam were derived by standardizing the genetic estimates to match the low-density lipoprotein cholesterol (LDL-C) lowering effect of BAS. CAD is defined as one of the following: MI >50% stenosis in at least one coronary vessel at angiography, history of percutaneous transluminal coronary angioplasty, or coronary artery bypass graft surgery, angina, or death caused by CAD.[24] Diff *P* represents the *P* value for difference between the predicted BAS effect on CAD outcomes using genetic data as compared with the effect of BAS on CAD outcome from the LRCCPPT and the difference between the effect of BAS compared with the effect from the CTT Trial, respectively. RCT indicates randomized controlled trial.

to fully understand the clinical efficacy and safety of colesevelam as compared with a placebo, alone or in combination with other lipid lowering agents.

The *ABCG5/8* genes and BAS act through related biological mechanisms. BAS bind to intestinal bile acids and are excreted through the feces, thus impeding the enterohepatic circulation of bile acid. This leads to an increase in bile acid synthesis and a subsequent decrease in plasma LDL-C levels.[51] Animal models have demonstrated that hepatic ABCG5/8 transporters are responsible for secreting multiple sterols in the bile, whereas intestinal transporters limit cholesterol absorption from the lumen and thus promote fecal excretion.[52,53] Overexpression of *ABCG5/8* genes in transgenic mice resulted in an increase in biliary cholesterol secretion, reduced cholesterol absorption, and increased hepatic cholesterol synthesis,[52] leading to a significant reduction in plasma cholesterol levels and atherosclerotic lesions. In addition, treatment with BAS has also been associated with reduced levels of fasting plasma glucose.[48] Although the underlying mechanism is unknown, it has been suggested that the binding of BAS to bile acids alters the GI tract glucose absorption.[54] In support of that hypothesis, studies have also indicated that gastric bypass surgery leads to an increase in glucose metabolism as a result of an increase in bile acid concentration.[55] In our study, we did not observe an association of rs4299376 SNP with the changes in the levels of fasting glucose or HbA_{1c} and diabetes mellitus using data from the MAGIC and DIAGRAM Consortia ($P>0.05$ for all),[38,39] suggesting that this could be a beneficial pleiotropic effect specific to the pharmacological agent. Genetic mutations of *ABCG5/8* have also been associated with sitosterolemia, a rare genetic disorder resulting in increased intestinal absorption, decreased biliary excretion of dietary sterols, hypercholesterolemia, and atherosclerosis. BAS treatment lowers blood levels of dietary sterols[56,57] and is recommended for patients with sitosterolemia. Teupser et al (2010) reported that common *ABCG5/8* polymorphisms lower phytosterol levels as well as CVD risk,[58] again confirming the similarity between BAS treatment and the effect of rs4299376. Taken together, these results confirm the similarity between BAS treatment and the effect of rs4299376. Therefore, our genetic results illustrate that inhibition of intestinal cholesterol absorption may provide a valuable therapeutic target for the prevention of CVD.

A few limitations of our study warrant discussion. First, Mendelian randomization analyses require some assumptions to be met for the analysis to be valid, and these include the following: the genetic variant is associated with the exposure of interest, the genetic variant is independent of confounders, and the genetic variant is independent of the outcome given the exposure and confounding factors.[12] Although the rs4299376 SNP acts through a similar functional pathway as BAS, we cannot exclude the possibility of pleiotropic effects of the genetic variant or off-target effects of the drug. For instance, both are involved in the absorption of dietary sterol, which may be a key mediator of their CAD protective effect. Second, we were unable to assess the effect of ethnicity on BAS efficacy because of the lack of reported data. Third, we found that the effect of colesevelam on triglycerides predicted by genetic data was statistically different from the pharmacological effect. Nonetheless, the predicted effect was weak (8.94 mg/dL [95% CI: 15.5, 2.32]) and should not affect CAD risk estimates because the effect size of triglycerides is modest in comparison with other CAD risk factors.[59] Furthermore, our meta-analysis may have been underpowered to detect any change because triglycerides are highly clinically variable. However, the effects on TC and HDL-C predicted from genetic data were consistent with estimates from the meta-analysis. Fourth, the protective effect of BAS on CAD was larger in the Mendelian randomization analysis as compared with the reported trend from LRCCPPT and estimates derived from the CTT. Although the differences in estimates were not statistically different, this may be because of the observation that rs4299376 carriers have a lifelong exposure to lower levels of LDL-C. Finally, the predicted side effects of BAS therapy using a Mendelian randomization analysis have not been addressed and further research may be required.

In summary, this systematic review, meta-analysis, and large-scale Mendelian randomization analysis illustrates that

pharmacological inhibition of intestinal cholesterol absorption may reduce the risk of major cardiovascular events. Comparisons of genetic association studies and clinical trials of colesevelam support the potential use of BAS as a second line therapy to reduce LDL-C in the prevention of CAD. Our results point to the need for large-scale randomized trials to fully assess the efficacy and safety of BAS treatment on CVD, as well as their effect when combined with other lipid lowering agents, such as statins.

Acknowledgments

We are thankful to all the participants having agreed to contribute to this project. Data on coronary artery disease have been contributed by CARDIoGRAMplusC4D investigators and have been downloaded from www.CARDIOGRAMPLUSC4D.ORG. Data on plasma lipid levels have been contributed by Global Lipids Genetic Consortium investigators and have been downloaded from http://www.sph.umich.edu/csg/abecasis/public/lipids2010/.

Sources of Funding

G. Paré is receiving support from Canada Research Chair in Genetic and Molecular Epidemiology, CISCO Professorship in Integrated Health Systems, and grant support from Canadian Institutes of Health Research (MOP-106715).

Disclosures

None.

References

1. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics–2013 update: a report from the American Heart Association. *Circulation*. 2013;127:e6–e245. doi: 10.1161/CIR.0b013e31828124ad.
2. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al; American College of Cardiology/American Heart Association Task Force on Practice Guidelines. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2014;129(25 Suppl 2):S1–S45. doi: 10.1161/01.cir.0000437738.63853.7a.
3. Taylor F, Huffman MD, Macedo AF, Moore TH, Burke M, Davey Smith G, et al. Statins for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev*. 2013;1:CD004816. doi: 10.1002/14651858.CD004816.pub5.
4. Davidson MH, Maki KC, Pearson TA, Pasternak RC, Deedwania PC, McKenney JM, et al. Results of the National Cholesterol Education (NCEP) Program Evaluation ProjecT Utilizing Novel E-Technology (NEPTUNE) II survey and implications for treatment under the recent NCEP Writing Group recommendations. *Am J Cardiol*. 2005;96:556–563. doi: 10.1016/j.amjcard.2005.04.019.
5. Abramson JD, Rosenberg HG, Jewell N, Wright JM. Should people at low risk of cardiovascular disease take a statin? *BMJ*. 2013;347:f6123.
6. Insull W Jr. Clinical utility of bile acid sequestrants in the treatment of dyslipidemia: a scientific review. *South Med J*. 2006;99:257–273. doi: 10.1097/01.smj.0000208120.73327.db.
7. Steinmetz KL, Schonder KS. Colesevelam: potential uses for the newest bile resin. *Cardiovasc Drug Rev*. 2005;23:15–30.
8. The Lipid Research Clinics Coronary Primary Prevention Trial results. I. Reduction in incidence of coronary heart disease. *JAMA*. 1984;251:351–364.
9. Andrade SE, Walker AM, Gottlieb LK, Hollenberg NK, Testa MA, Saperia GM, et al. Discontinuation of antihyperlipidemic drugs–do rates reported in clinical trials reflect rates in primary care settings? *N Engl J Med*. 1995;332:1125–1131. doi: 10.1056/NEJM199504273321703.
10. Watts GF, Lewis B, Brunt JN, Lewis ES, Coltart DJ, Smith LD, et al. Effects on coronary artery disease of lipid-lowering diet, or diet plus cholestyramine, in the St Thomas' Atherosclerosis Regression Study (STARS). *Lancet*. 1992;339:563–569.
11. Brensike JF, Levy RI, Kelsey SF, Passamani ER, Richardson JM, Loh IK, et al. Effects of therapy with cholestyramine on progression of coronary arteriosclerosis: results of the NHLBI Type II Coronary Intervention Study. *Circulation*. 1984;69:313–324.
12. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med*. 2008;27:1133–1163. doi: 10.1002/sim.3034.
13. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol*. 2003;32:1–22.
14. Sofat R, Hingorani AD, Smeeth L, Humphries SE, Talmud PJ, Cooper J, et al. Separating the mechanism-based and off-target actions of cholesteryl ester transfer protein inhibitors with CETP gene polymorphisms. *Circulation*. 2010;121:52–62. doi: 10.1161/CIRCULATIONAHA.109.865444.
15. Hingorani AD, Casas JP. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. *Lancet*. 2012;379:1214–1224.
16. Schmitz G, Langmann T, Heimerl S. Role of ABCG1 and other ABCG family members in lipid metabolism. *J Lipid Res*. 2001;42:1513–1520.
17. Brown JM, Yu L. Opposing Gatekeepers of Apical Sterol Transport: Niemann-Pick C1-Like 1 (NPC1L1) and ATP-Binding Cassette Transporters G5 and G8 (ABCG5/ABCG8). *Immunol Endocr Metab Agents Med Chem*. 2009;9:18–29. doi: 10.2174/187152209788009797.
18. Oram JF, Vaughan AM. ATP-Binding cassette cholesterol transporters and cardiovascular disease. *Circ Res*. 2006;99:1031–1043. doi: 10.1161/01.RES.0000250171.54048.5c.
19. IBC 50K CAD Consortium. Large-scale gene-centric analysis identifies novel variants for coronary artery disease. *PLoS Genet*. 2011;7:e1002260. doi: 10.1371/journal.pgen.1002260.
20. Davidson MH, Toth P, Weiss S, McKenney J, Hunninghake D, Isaacsohn J, et al. Low-dose combination therapy with colesevelam hydrochloride and lovastatin effectively decreases low-density lipoprotein cholesterol in patients with primary hypercholesterolemia. *Clin Cardiol*. 2001;24:467–474.
21. Cederberg H, Gylling H, Miettinen TA, Paananen J, Vangipurapu J, Pihlajamäki J, et al. Non-cholesterol sterol levels predict hyperglycemia and conversion to type 2 diabetes in Finnish men. *PLoS One*. 2013;8:e67406. doi: 10.1371/journal.pone.0067406.
22. Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Genet*. 2013;45:25–33.
23. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature*. 2010;466:707–713. doi: 10.1038/nature09270.
24. Samani NJ, Deloukas P, Erdmann J, Hengstenberg C, Kuulasmaa K, McGinnis R, et al. Large scale association analysis of novel genetic loci for coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2009;29:774–780.
25. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al; Cholesterol Treatment Trialists' (CTT) Collaborators. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet*. 2005;366:1267–1278. doi: 10.1016/S0140-6736(05)67394-1.
26. Betteridge DJ, Bhatnager D, Bing RF, Durrington PN, Evans GR, Flax H, et al. Treatment of familial hypercholesterolaemia. United Kingdom lipid clinics study of pravastatin and cholestyramine. *BMJ*. 1992;304:1335–1338.
27. Comparative efficacy and safety of pravastatin and cholestyramine alone and combined in patients with hypercholesterolemia. Pravastatin Multicenter Study Group II. *Arch Intern Med*. 1993;153:1321–1329.
28. Wiklund O, Angelin B, Fager G, Eriksson M, Olofsson SO, Berglund L, et al. Treatment of familial hypercholesterolaemia: a controlled trial of the effects of pravastatin or cholestyramine therapy on lipoprotein and apolipoprotein levels. *J Intern Med*. 1990;228:241–247.
29. Bays HE, Goldberg RB, Truitt KE, Jones MR. Colesevelam hydrochloride therapy in patients with type 2 diabetes mellitus treated with metformin: glucose and lipid effects. *Arch Intern Med*. 2008;168:1975–1983. doi: 10.1001/archinte.168.18.1975.
30. Davidson MH, Dillon MA, Gordon B, Jones P, Samuels J, Weiss S, et al. Colesevelam hydrochloride (cholestagel): a new, potent bile acid sequestrant associated with a low incidence of gastrointestinal side effects. *Arch Intern Med*. 1999;159:1893–1900.
31. Devaraj S, Autret B, Jialal I. Effects of colesevelam hydrochloride (WelChol) on biomarkers of inflammation in patients with mild hypercholesterolemia. *Am J Cardiol*. 2006;98:641–643. doi: 10.1016/j.amjcard.2006.03.043.
32. Handelsman Y, Goldberg RB, Garvey WT, Fonseca VA, Rosenstock J, Jones MR, et al. Colesevelam hydrochloride to treat hypercholesterolemia and improve glycemia in prediabetes: a randomized, prospective study. *Endocr Pract*. 2010;16:617–628. doi: 10.4158/EP10129.OR.

33. Hunninghake D, Insull W Jr, Toth P, Davidson D, Donovan JM, Burke SK. Coadministration of colesevelam hydrochloride with atorvastatin lowers LDL cholesterol additively. *Atherosclerosis*. 2001;158:407–416.
34. Insull W Jr, Toth P, Mullican W, Hunninghake D, Burke S, Donovan JM, et al. Effectiveness of colesevelam hydrochloride in decreasing LDL cholesterol in patients with primary hypercholesterolemia: a 24-week randomized controlled trial. *Mayo Clin Proc*. 2001;76:971–982. doi: 10.4065/76.10.971.
35. Knapp HH, Schrott H, Ma P, Knopp R, Chin B, Gaziano JM, et al. Efficacy and safety of combination simvastatin and colesevelam in patients with primary hypercholesterolemia. *Am J Med*. 2001;110:352–360.
36. Rosenstock J, Fonseca VA, Garvey WT, Goldberg RB, Handelsman Y, Abby SL, et al. Initial combination therapy with metformin and colesevelam for achievement of glycemic and lipid goals in early type 2 diabetes. *Endocr Pract*. 2010;16:629–640. doi: 10.4158/EP10130.OR.
37. Zieve FJ, Kalin MF, Schwartz SL, Jones MR, Bailey WL. Results of the glucose-lowering effect of WelChol study (GLOWS): a randomized, double-blind, placebo-controlled pilot study evaluating the effect of colesevelam hydrochloride on glycemic control in subjects with type 2 diabetes. *Clin Ther*. 2007;29:74–83. doi: 10.1016/j.clinthera.2007.01.003.
38. Morris AP, Voight BF, Teslovich TM, Ferreira T, Segrè AV, Steinthorsdottir V, et al; Wellcome Trust Case Control Consortium; Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) Investigators; Genetic Investigation of ANthropometric Traits (GIANT) Consortium; Asian Genetic Epidemiology Network–Type 2 Diabetes (AGEN-T2D) Consortium; South Asian Type 2 Diabetes (SAT2D) Consortium; DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nat Genet*. 2012;44:981–990. doi: 10.1038/ng.2383.
39. Scott RA, Lagou V, Welch RP, Wheeler E, Montasser ME, Luan J, et al; DIAbetes Genetics Replication and Meta-analysis (DIAGRAM) Consortium. Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. *Nat Genet*. 2012;44:991–1005. doi: 10.1038/ng.2385.
40. Soranzo N, Sanna S, Wheeler E, Gieger C, Radke D, Dupuis J, et al; WTCCC. Common variants at 10 genomic loci influence hemoglobin A_1© levels via glycemic and nonglycemic pathways. *Diabetes*. 2010;59:3229–3239. doi: 10.2337/db10-0502.
41. Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al; MAGIC; Procardis Consortium. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet*. 2010;42:937–948. doi: 10.1038/ng.686.
42. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*. 2011;478:103–109.
43. Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, et al. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. *N Engl J Med*. 2011;365:2255–2267.
44. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, et al; ILLUMINATE Investigators. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med*. 2007;357:2109–2122. doi: 10.1056/NEJMoa0706628.
45. HPS2-THRIVE randomized placebo-controlled trial in 25 673 high-risk patients of ER niacin/laropiprant: trial design, pre-specified muscle and liver outcomes, and reasons for stopping study treatment. *Eur Heart J*. 2013;34:1279–1291.
46. Davidson MH. The use of colesevelam hydrochloride in the treatment of dyslipidemia: a review. *Expert Opin Pharmacother*. 2007;8:2569–2578. doi: 10.1517/14656566.8.15.2569.
47. Donovan JM, Stypinski D, Stiles MR, Olson TA, Burke SK. Drug interactions with colesevelam hydrochloride, a novel, potent lipid-lowering agent. *Cardiovasc Drugs Ther*. 2000;14:681–690.
48. Ooi CP, Loke SC. Colesevelam for type 2 diabetes mellitus. *Cochrane Database Syst Rev*. 2012;12:CD009361. doi: 10.1002/14651858.CD009361.pub2.
49. Cannon CP. Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, Darius H, Lewis BS, Ophuis TO, Jukema JW, De Ferrari GM, Ruzyllo W, De Lucca P, Im K, Bohula EA, Reist C, Wiviott SD, Tershakovec AM, Musliner TA, Braunwald E, Califf RM; IMPROVE-IT Investigators. Ezetimibe added to statin therapy after acute coronary syndromes. *N Engl J Med*. 2015;372:2387–2397.
50. Stitziel NO, Won HH, Morrison AC, Peloso GM, Do R, Lange LA, et al. Inactivating mutations in NPC1L1 and protection from coronary heart disease. *N Engl J Med*. 2014;371:2072–2082.
51. Bays H, Dujovne C. Colesevelam HCl: a non-systemic lipid-altering drug. *Expert Opin Pharmacother*. 2003;4:779–790. doi: 10.1517/14656566.4.5.779.
52. Yu L, Li-Hawkins J, Hammer RE, Berge KE, Horton JD, Cohen JC, et al. Overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. *J Clin Invest*. 2002;110:671–680. doi: 10.1172/JCI16001.
53. Yu L, Hammer RE, Li-Hawkins J, Von Bergmann K, Lutjohann D, Cohen JC, et al. Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. *Proc Natl Acad Sci U S A*. 2002;99:16237–16242. doi: 10.1073/pnas.252582399.
54. Staels B, Fonseca VA. Bile acids and metabolic regulation: mechanisms and clinical responses to bile acid sequestration. *Diabetes Care*. 2009;32 Suppl 2:S237–S245. doi: 10.2337/dc09-S355.
55. Jørgensen NB, Dirksen C, Bojsen-Møller KN, Kristiansen VB, Wulff BS, Rainteau D, et al. Improvements in glucose metabolism early after gastric bypass surgery are not explained by increases in total bile acids and fibroblast growth factor 19 concentrations. *J Clin Endocrinol Metab*. 2015;100:E396–E406. doi: 10.1210/jc.2014-1658.
56. Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, et al. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science*. 2000;290:1771–1775.
57. Lu K, Lee MH, Hazard S, Brooks-Wilson A, Hidaka H, Kojima H, et al. Two genes that map to the STSL locus cause sitosterolemia: genomic structure and spectrum of mutations involving sterolin-1 and sterolin-2, encoded by ABCG5 and ABCG8, respectively. *Am J Hum Genet*. 2001;69:278–290.
58. Teupser D, Baber R, Ceglarek U, Scholz M, Illig T, Gieger C, et al. Genetic regulation of serum phytosterol levels and risk of coronary artery disease. *Circ Cardiovasc Genet*. 2010;3:331–339. doi: 10.1161/CIRCGENETICS.109.907873.
59. Miller M, Stone NJ, Ballantyne C, Bittner V, Criqui MH, Ginsberg HN, et al; American Heart Association Clinical Lipidology, Thrombosis, and Prevention Committee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Arteriosclerosis, Thrombosis and Vascular Biology; Council on Cardiovascular Nursing; Council on the Kidney in Cardiovascular Disease. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. *Circulation*. 2011;123:2292–2333. doi: 10.1161/CIR.0b013e3182160726.

CLINICAL PERSPECTIVE

Statins are the primary therapeutic agents in the prevention of coronary artery disease (CAD), but may not be well tolerated or effective in all patients. Other lipid lowering agents, such as bile acid sequestrants (BAS), may be used as an alternative or in combination with statins. However, only a few underpowered BAS clinical trials have demonstrated a modest trend toward reduced risk of clinical outcomes. We conducted a meta-analysis of cholestyramine, colestipol, and colesevelam clinical trials to assess the effect of BAS on plasma lipid levels and CAD outcomes. We then applied the principles of Mendelian randomization to estimate the effect of BAS on risk of CAD by using the effects of the rs4299376 SNP (*ABCG5/ABCG8*) on low-density lipoprotein cholesterol and CAD. We demonstrated that the predicted reduction in risk of CAD by cholestyramine (OR 0.63, 95% CI 0.52–0.77; $P=6.3\times10^{-6}$) and colesevelam (OR 0.64, 95% CI 0.52–0.79, $P=4.3\times10^{-5}$) is consistent with the nonsignificant trends observed in clinical trials. These results have clinical implications because they suggest that BAS may provide an effective second line therapy among patients who are unable to tolerate statin therapy or in whom statins are ineffective in reducing low-density lipoprotein cholesterol. This study also highlights the use of Mendelian randomization in drug target validation and to complement clinical trial data.

SUPPLEMENTAL MATERIALS

SUPPLEMENTAL METHODS

Systematic Review and Meta-Analysis

Eligibility Criteria

Types of studies: Randomized, double-blinded, placebo controlled clinical trials (RCTs) that compared bile acid sequestrant (BAS) treatment with placebo. There were no restrictions based on publication status or publication date; however, only studies published in English were considered.

Type of patients: Only patients aged ≥ 18 years were considered for this review.

Type of Intervention: RCTs that compared the effects of BAS (i.e. 24 g daily cholestyramine, 5 g/d colestipol, and 3.75 g/d colesevelam) with placebo or no treatment. There were no restrictions based on the frequency, dosage, length or duration of the BAS intervention.

Types of Outcome Measures:

Primary outcome measures include:

1. Cardiovascular mortality;
2. Myocardial infarction (MI); and
3. Baseline and endpoint mean values or the absolute treatment difference in the intervention and placebo arms for the change in low density lipoprotein cholesterol (LDL-C) levels.

Studies with at least one of these primary outcomes were considered.

Secondary outcome measures include:

1. Baseline and endpoint mean values or the absolute treatment difference in the intervention and placebo arms for the change in high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglycerides, apolipoprotein A1 (apoA), and apolipoprotein B (apoB).

Information sources

A structured literature search was performed by identifying studies through electronic databases, hand searching reference lists, consulting with field experts and pharmaceutical companies, and scanning trial registries. This search was applied to PubMed (1946 to 2014 in Ovid).

Search

The following terms were used to search all clinical trial registries and databases: cholestyramine; colestipol; colesevelam HCl; placebo; and randomized controlled trials. Where possible, authors of relevant publications were contacted to provide additional information and details about outstanding issues.

Study Selection and Data Items

Based on the results of the search strategy, titles and abstracts for each reference were examined independently by two reviewers (MD and SR). Relevant studies obtained from the full-text screening phase were reviewed for methodological quality and disagreements were resolved through discussion or consultation with a clinician (GP). The following information was extracted from each included trial: (1) characteristics of the study participants (i.e. age, sex,

patient population); (2) characteristics of the study (i.e. study design, sample size, median follow-up period); (3) characteristics of the intervention (i.e. dose and frequency of the intervention); and (4) characteristics of the outcome measures (including cardiovascular mortality, MI, and mean change in LDL-C, HDL-C, TC, triglycerides, apoA and apoB).

Data collection process

The two reviewers independently extracted data from the included studies using data collection forms. When methodological information could not be obtained from a publication, the author was contacted for further comment. All forms used in this systematic review were subject to pilot-testing using ten randomly selected studies. Data entry was performed independently by one reviewer (SR) and cross-referenced by the other reviewer (MD). Any discrepancies between the two reviewers were documented and the forms were changed accordingly.

Summary measures

For continuous traits, studies that reported median values were converted to an equivalent mean value and the corresponding standard deviation values were calculated by dividing the interquartile range by 1.35. If studies did not report the standard deviation, it was calculated by multiplying the standard error by the square root of the sample size. The mean age across RCTs was reported as the sample size weighted mean. Where data for LDL-C, HDL-C and TC were available in units of mmol/L, they were converted to mg/dL using a multiplication factor of 38.66. Triglycerides, and apoA and apoB were similarly converted using a multiplication factor of 88.6 and 100, respectively. The mean change-from-baseline in plasma lipid levels in the BAS intervention group were compared to the mean differences in the placebo group with the 95%

confidence interval (CI) and p-value as a measure of uncertainty. For binary outcomes, the treatment effect was expressed as an odds ratio (OR) with the 95% CI and p-value. Meta-analyses were performed using an inverse variance random effect meta-analysis.

Synthesis of results

Heterogeneity was assessed using the chi-square statistic (χ^2) and inconsistency (I^2) was measured by assessing the percentage of total variation of the effects of BAS across studies due to heterogeneity. A low p-value ($p<0.10$) or I^2 test statistic of > 30% provided evidence of heterogeneity of intervention effects. If these estimates gave rise to sufficient evidence of heterogeneity than attempts were made to explain these differences.

Additional Analyses

To explain any evidence of heterogeneity, subgroup analyses were conducted based on the characteristics of the participants (i.e. presence of hyperlipidaemia or type 2 diabetes mellitus) and the study interventions (i.e. length of follow-up). Sensitivity analyses were pre-specified and were used to test the robustness of the pooled results. Unless otherwise specified, a correlation coefficient (*r*) of 0.5 for the difference in the mean change from baseline was assumed for all analyses. Thus the *r* was varied by 0.3 and 0.7 for all the relevant studies to determine if this altered the reported estimates [1].

Simulation Statistical Analysis

Simulations were performed to predict the effect of 24 g/d cholestyramine on plasma lipid profiles (HDL-C, TC, triglycerides, apoA and apoB) using the known genetic associations of

rs4299376 SNP with lipids fractions. To do so, we adapted the method from Sofat et al[2] to match the genetic effects to the effect of cholestyramine 24 g/d on LDL-C, taking into account the uncertainty of both the genetic and drug effect estimates. Random numbers were selected from the normal distributions of the change in LDL-C for the pharmacological and genetic effect (i.e. fixing the mean and standard deviation of each distribution to their respective estimated values). In order to validate whether the rs4299376 SNP had a similar effect on plasma lipid profiles as cholestyramine, the predicted effects of cholestyramine on plasma levels of HDL-C, TC and triglycerides were estimated using genetic data. These predicted estimates were then compared to known effects of cholestyramine on the same lipids fractions from clinical data. 10,000 simulations were performed to generate the distribution of HDL-C, TC and triglycerides assuming each allele has the same predicted effect as cholestyramine on LDL-C, and the mean effect and 95% CI were calculated. The p-value for the difference between the predicted effect of cholestyramine and the observed effects of BAS on lipid levels were calculated by comparing the randomly generated point estimate of the effect of cholestyramine to the randomly generated point estimate of the predicted effect of the drug. Next, the effect of 24 g/d cholestyramine on the risk of cardiovascular outcomes was predicted using data on genetic association of rs4299376 with CAD and compared to the effect of cholestyramine on CAD from the only outcome trial of cholestyramine, LRCCPPT[3]. The predicted drug effect was compared to the observed effect of a comparable dose of cholestyramine on the risk of CVD outcomes using a z-test. As a sensitivity analysis, the predicted effect of cholestyramine on CAD was also estimated using data from the CTT[4]. This estimate was similarly compared to the cardiovascular outcomes reported in the LRCCPPT in order to compare the predicted effect of BAS with statin use using a z-test. These analyses were also repeated using the summary effect of 3.75 g/d of colesevelam.

Results

Study Selection

A total of 19 studies were identified for inclusion in this review. The structured literature search of PubMed databases derived a total of 420 citations. Of these, 360 studies were discarded because after reviewing the abstracts it appeared that these papers clearly did not meet our inclusion criteria. The full-text of the remaining 60 citations were examined in more detail. It appeared that 40 articles did not meet the inclusion criteria. Of the included articles, there were six cholestyramine RCTs[3, 5-9], three colestipol RCTs[10-12] and 10 colesevelam RCTs[13-21] with a total of 7,021 study participants. Supplemental Figure1 illustrates the flow diagram of the study selection process.

Randomized Controlled Trials of Colestipol

We identified three RCTs with a total of 398 participants with hyperlipidemia (mean age 52 years, 44% women)[10-12] (Supplemental Table 1). Owing to the lack of reported data and differences in study dose, we did not pool the reported effect of colestipol on plasma lipid levels.

Additional Analyses

We were unable to conduct subgroup analyses in order to explore the presence of heterogeneity among the pooled estimates of 24 g/d cholestyramine and 3.75 g/d colesevelam on the mean change in plasma lipid levels due to a lack of reported data. Therefore, to account for the high degree of heterogeneity in the pooled estimates of cholestyramine, the effect estimates of the

mean change in LDL-C and TC from the LRCCPPT trial[3] will be used as a surrogate since it was the only outcome trial.

To test the robustness of the main findings, the *r* of the mean change from baseline in the 24 g/d cholestyramine and the 3.75 g/d colesevelam meta-analyses were varied. Assuming an *r* of 0.3 and 0.7 did not demonstrate any difference in the reported treatment effects of cholestyramine (Supplemental Figure 3 and 4) or colesevelam (Supplemental Figure 5 and 6). However, assuming an r=0.3 within the cholestyramine meta-analysis resulted in a reduction of the high degree of heterogeneity in the pooled LDL-C estimates (P for heterogeneity =1.70×10^{-4}) while an r=0.7 significantly increased the presence of heterogeneity (heterogeneity P-value:2.10×10^{-9}). Similar results were also obtained for the treatment effects of colesevelam.

SUPPLEMENTAL TABLES

Supplemental Table #1: Studies contributing to the colestipol meta-analysis

Author & Date	Patient Population	Sample size	Intervention	Comparison	Follow-Up	Age (Mean, SD)	Women (%)	European (%)	LDL-C (mg/dL)*	
									Baseline	Endpoint
Hunninghake 1995[10]	Hyperlipidemia	196	Colestipol (2 g; 4 g; 8 g; 16 g)	Placebo	8 weeks	56.2 (NR)	101 (52)	NR	190.0(NR)	141.3(NR)
Simons 1992[11]	Hyperlipidemia	61	Colestipol (5 g); Colestipol (10 g) & each with 6 weeks of placebo; 6 weeks of simvaslatin (20 mg); 6 weeks of simvastatin (40 mg)	Placebo with 6 weeks of placebo; 6 weeks of simvaslatin (20 mg); 6 weeks of simvastatin (40 mg)	18 weeks	45.3 (19)	24 (39)	26 (43)	303.1(77.7)	266.7 (NR)
Superko 1992[12]	Hyperlipidemia	141	Colestipol (5g/d; 10g/d; 15g/d)	Placebo	12 weeks	49(12)	49 (35)	NR	168.0(12.0)	122.8(NR)

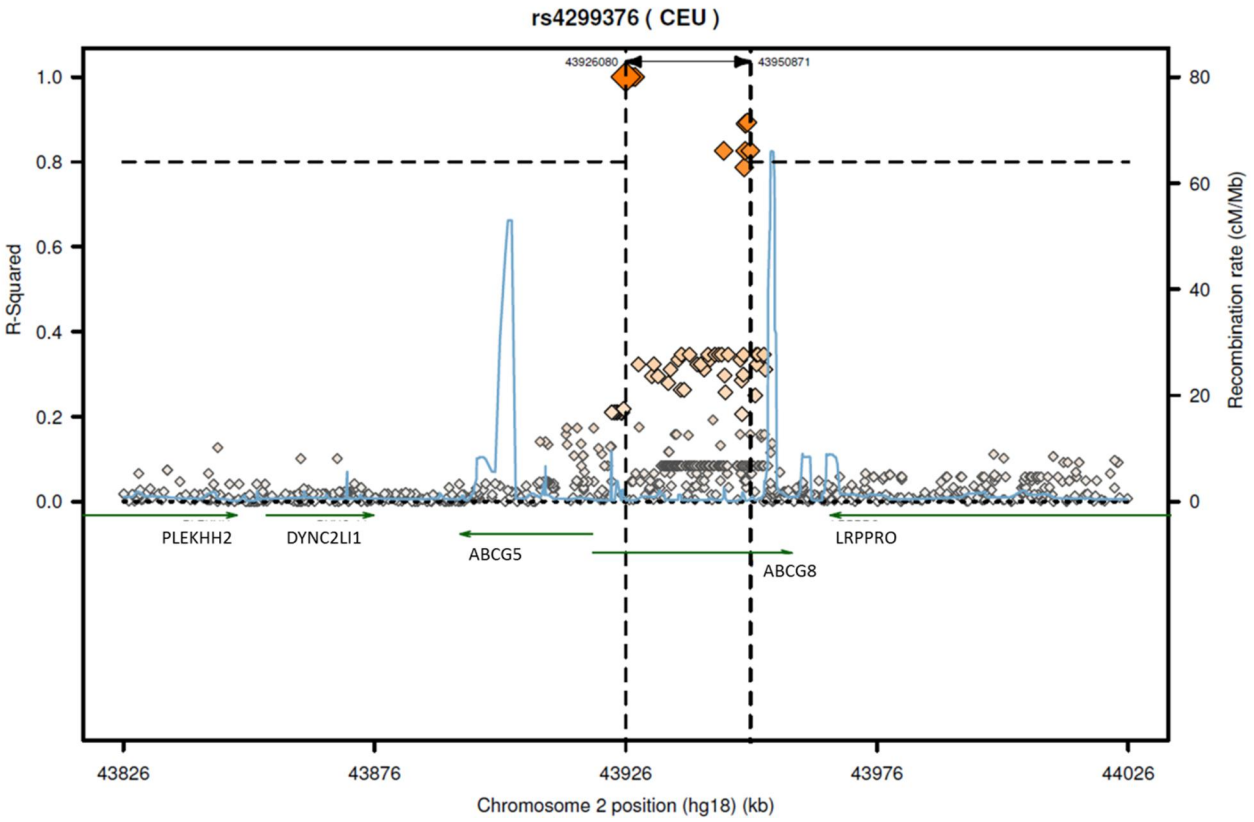
*Refers to the highest single BAS dose reported in the study; NR: not reported

Supplemental Table #2: The association of rs4299376 SNP (*ABCG5/8*) and the risk of LDL-C, HDL-C, TC, TG, diabetes, glycated hemoglobin (HbA_{1c}), fasting glucose (FG), systolic blood pressure (SBP), diastolic blood pressure (DBP) and body mass index (BMI).

Trait	Effect Allele	Other Allele	Effect Estimate	Standard Error	P-Value
LDL-C	T	G	-2.75	0.19898	1.73E-47
HDL-C	T	G	0.05	0.07143	0.212
TC	T	G	-3.01	0.21939	4.00E-45
TG	T	G	-1.08	0.36735	0.003
FG	G	T	0.00088	0.0026	0.737689
HbA_{1c}	T	G	-0.0051	0.004	0.199
Diabetes	G	T	-0.00738	0.016336	0.65164
SBP	G	T	0.024683	0.112445	0.826253
DBP	G	T	0.00435	0.070956	0.951115
BMI	T	G	-0.0054	0.0064	0.4

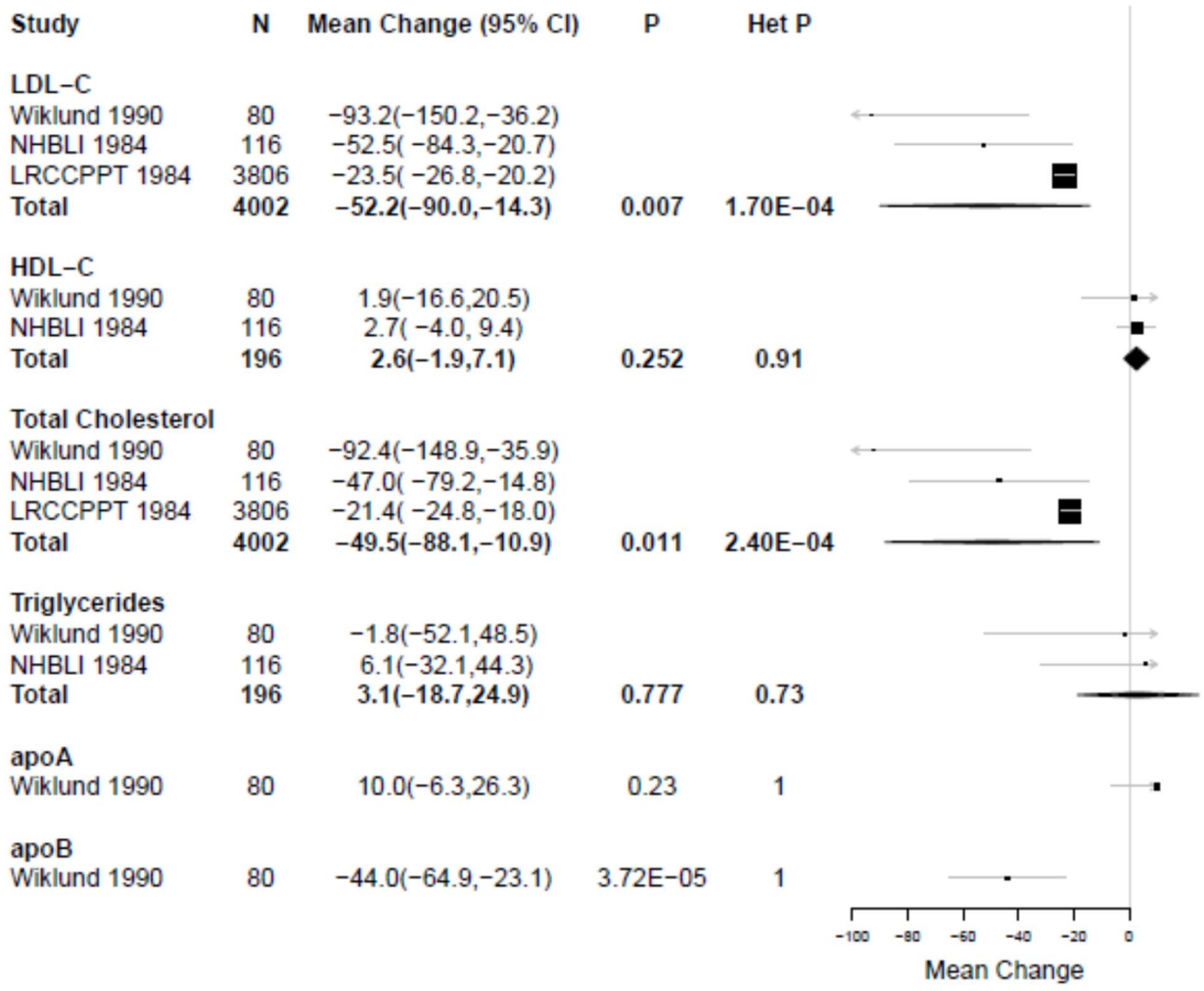
SUPPLEMENTAL FIGURES

Supplemental Figure #1: Regional LD Plot of rs4299376 (*ABCG5/ABCG8*).
Adapted from SNAP (Broad Institute) with data from the 1000 Genomes Pilot 1.



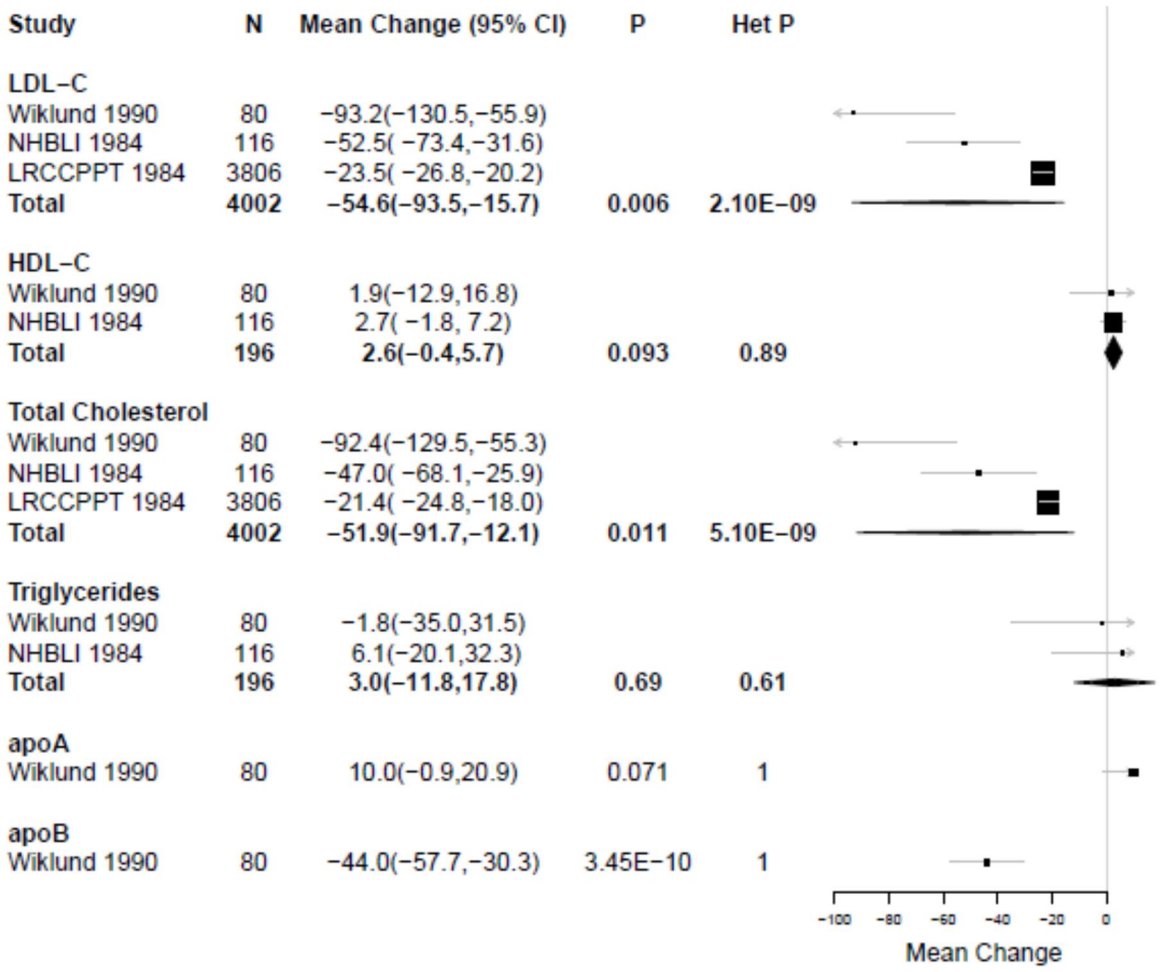
Supplemental Figure #2: Forest plot of the association of 24 g/d of cholestyramine treatment and the summary mean difference of LDL-C, HDL-C, total cholesterol, triglycerides, apoA and apoB assuming a correlation coefficient 0.3.

Het P refers to the heterogeneity p-value.



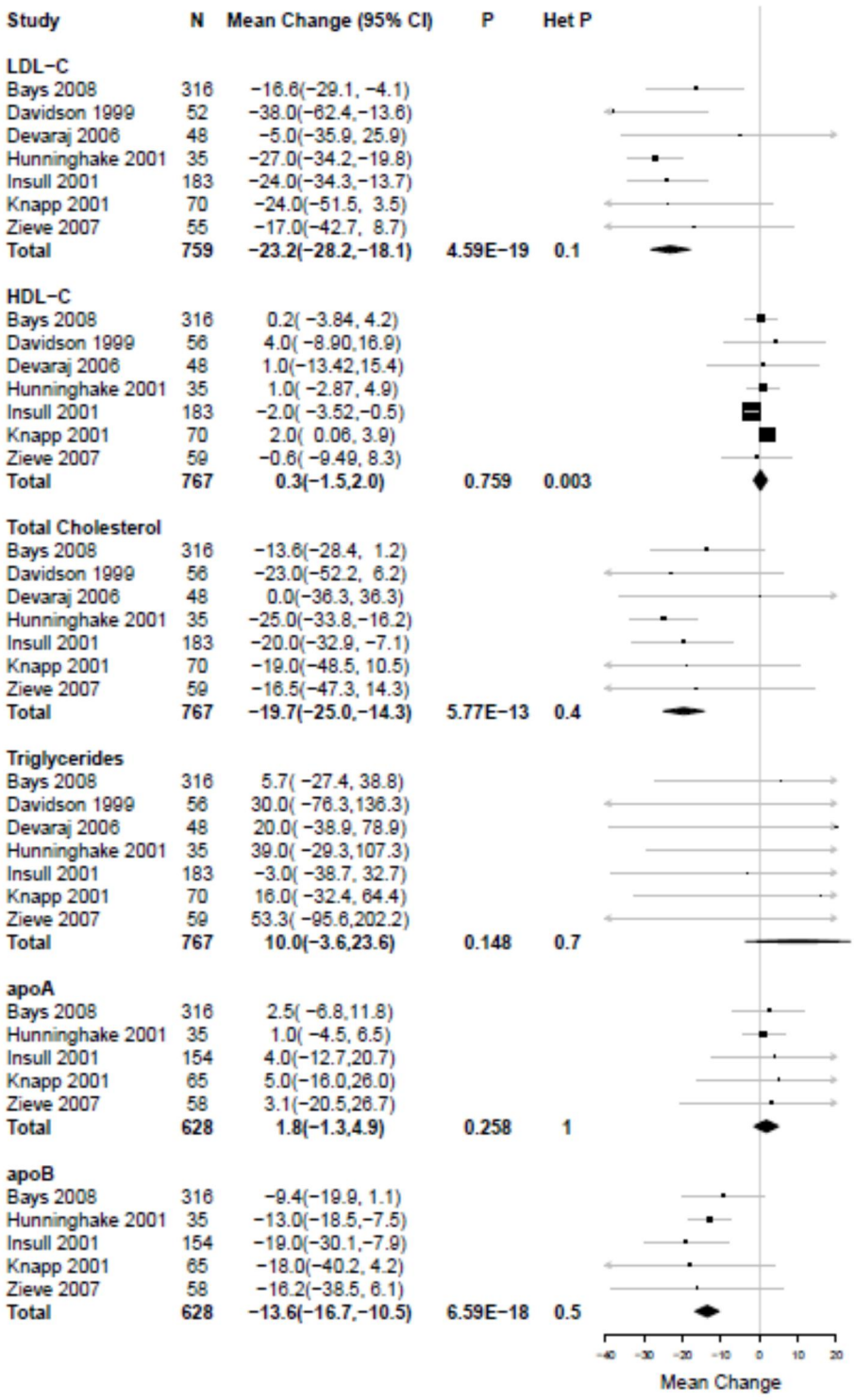
Supplemental Figure #3: Forest plot of the association of 24 g/d of cholestyramine treatment and the summary mean difference of LDL-C, HDL-C, total cholesterol, triglycerides, apoA and apoB assuming a correlation coefficient 0.7.

Het P refers to the heterogeneity p-value.



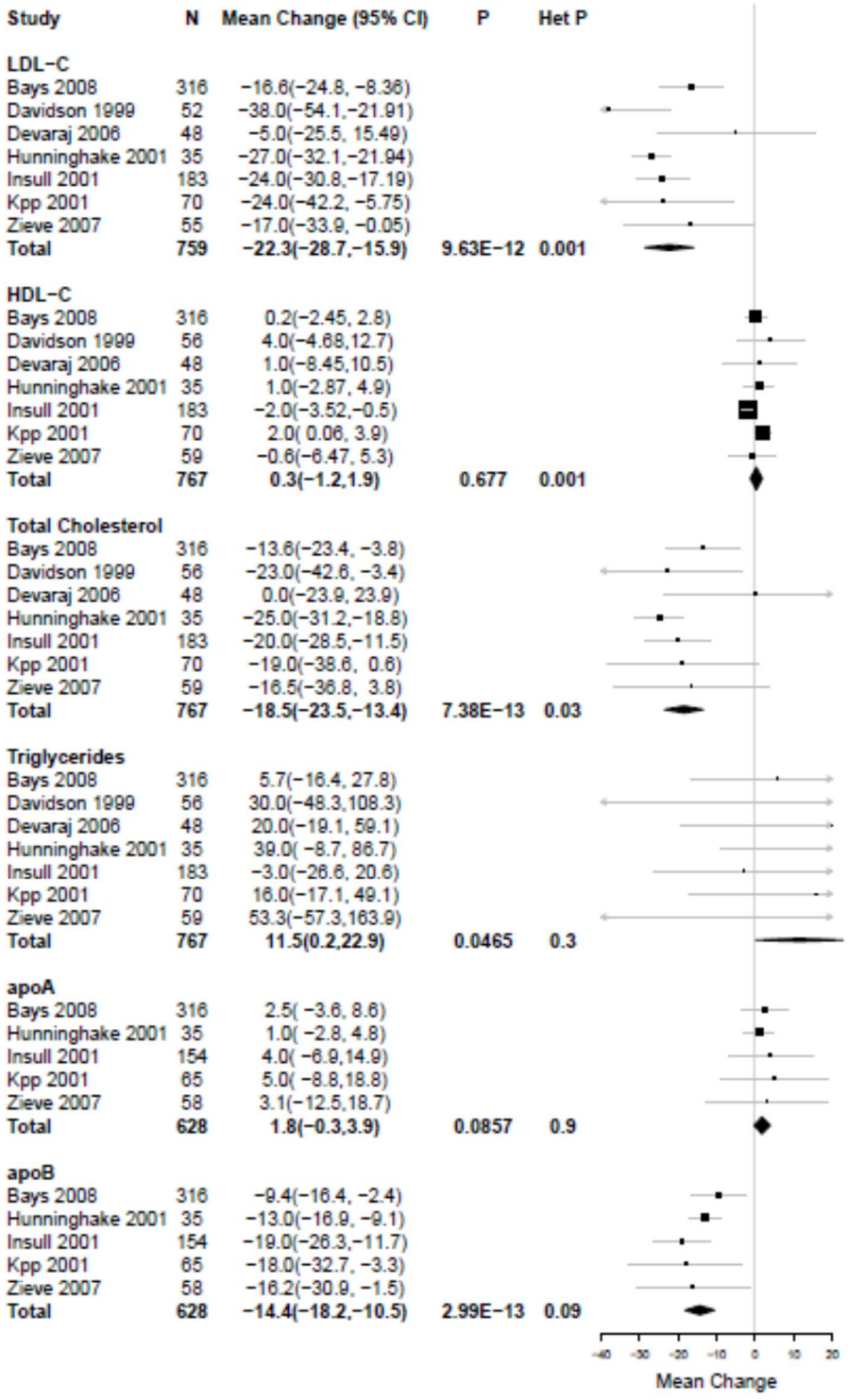
Supplemental Figure #4: Forest plot of the association of 3.75 g/d of colesevelam treatment and the summary mean difference of LDL-C, HDL-C, total cholesterol, triglycerides, apoA and apoB assuming a correlation coefficient 0.3.

Het P refers to the heterogeneity p-value.

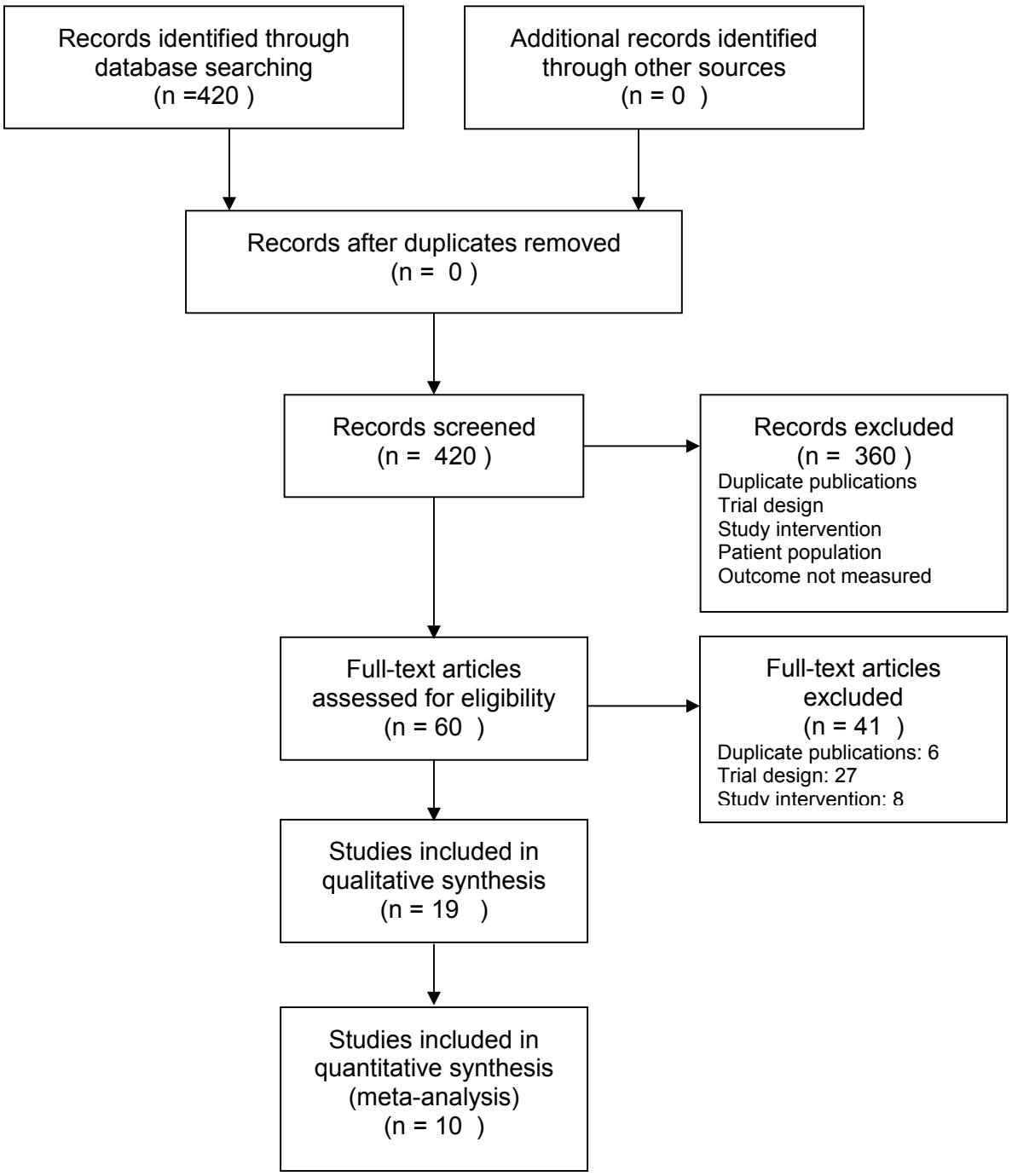


Supplemental Figure #5: Forest plot of the association of 3.75 g/d of colesevelam treatment and the summary mean difference of LDL-C, HDL-C, total cholesterol, triglycerides, apoA and apoB assuming a correlation coefficient 0.7.

Het P refers to the heterogeneity p-value.



Supplemental Figure #6: Study flow diagram.



List of CARDIoGRAMplusC4D authors

Panos Deloukas[1,126], Stavroula Kanoni[1,126], Christina Willenborg[2,126], Martin Farrall[3,4,126], Themistocles L Assimes[5,126], John R Thompson[6,126], Erik Ingelsson[7,126], Danish Saleheen[8–10,126], Jeanette Erdmann[2,126], Benjamin A Goldstein[5], Kathleen Stirrups[1], Inke R König[11], Jean-Baptiste Cazier[4], Åsa Johansson[12], Alistair S Hall[13], Jong-Young Lee[14], Cristen J Willer[15,16], John C Chambers[17], Tõnu Esko[18,19], Lasse Folkersen[20,21], Anuj Goel[3,4], Elin Grundberg[22], Aki S Havulinna[23], Weang K Ho[10], Jemma C Hopewell[24,25], Niclas Eriksson[12], Marcus E Kleber[26,27], Kati Kristiansson[23], Per Lundmark[28], Leo-Pekka Lyytikäinen[29,30], Suzanne Rafelt[31], Dmitry Shungin[32–34], Rona J Strawbridge[20,21], Gudmar Thorleifsson[35], Emmi Tikkanen[36,37], Natalie Van Zuydam[38], Benjamin F Voight[39], Lindsay L Waite[40], Weihua Zhang[17], Andreas Ziegler[11], Devin Absher[40], David Altshuler[41–44], Anthony J Balmforth[45], Inês Barroso[1,46], Peter S Braund[31,47], Christof Burgdorf[48], , Simone Claudi-Boehm[49], David Cox[50], Maria Dimitriou[51], Ron Do[41,43], CARDIOGENICS Consortium[52], DIAGRAM Consortium[52], Alex S F Doney[38], NourEddine El Mokhtari[53], Per Eriksson[20,21], Krista Fischer[18], Pierre Fontanillas[41], Anders Franco-Cereceda[54], Bruna Gigante[55], Leif Groop[56], Stefan Gustafsson[7], Jörg Hager[57], Göran Hallmans[58], Bok-Ghee Han[14], Sarah E Hunt[1], Hyun M Kang[59], Thomas Illig[60], Thorsten Kessler[48], Joshua W Knowles[5], Genovefa Kolovou[61], Johanna Kuusisto[62], Claudia Langenberg[63], Cordelia Langford[1], Karin Leander[55], Marja-Liisa Lokki[64], Anders Lundmark[28], Mark I McCarthy[3,65,66], Christa Meisinger[67], Olle Melander[56], Evelin Mihailov[19], Seraya Maouche[68], Andrew D Morris[38], Martina Müller-Nurasyid[69–72], MuTHER Consortium[52], Kjell Nikus[73], John F Peden[3], N William Rayner[3], Asif Rasheed[9], Silke Rosinger[74], Diana Rubin[53], Moritz P Rumpf[48], Arne Schäfer[75], Mohan Sivananthan[76,77], Ci Song[7], Alexandre F R Stewart[78,79], Sian-Tsung Tan[80], Gudmundur Thorgeirsson[81,82], C Ellen van der Schoot[83], Peter J Wagner[36,37], Wellcome Trust Case Control Consortium[52], George A Wells[78,79], Philipp S Wild[84,85], Tsun-Po Yang[1], Philippe Amouyel[86], Dominique Arveiler[87], Hanneke Basart[88], Michael Boehnke[59], Eric Boerwinkle[89], Paolo Brambilla[90], Francois Cambien[68], Adrienne L Cupples[91,92], Ulf de Faire[55], Abbas Dehghan[93], Patrick Diemert[94], Stephen E Epstein[95], Alun Evans[96], Marco M Ferrario[97], Jean Ferrières[98], Dominique Gauguier[3,99], Alan S Go[100], Alison H Goodall[31,47], Villi Gudnason[81,101], Stanley L Hazen[102], Hilma Holm[35], Carlos Iribarren[100], Yangsoo Jang[103], Mika Kähönen[104], Frank Kee[105], Hyo-Soo Kim[106], Norman Klopp[60], Wolfgang Koenig[107], Wolfgang Kratzer[108], Kari Kuulasmaa[23], Markku Laakso[62], Reijo Laaksonen[108], Ji-Young Lee[14], Lars Lind[28], Willem H Ouwehand[1,109,110], Sarah Parish[24,25], Jeong E Park[111], Nancy L Pedersen[7], Annette Peters[67,112], Thomas Quertermous[5], Daniel J Rader[113], Veikko Salomaa[23], Eric Schadt[114], Svati H Shah[115,116], Juha Sinisalo[117], Klaus Stark[118], Kari Stefansson[35,81], David- Alexandre Trégouët[68], Jarmo Virtamo[23], Lars Wallentin[12], Nicholas Wareham[63], Martina E Zimmermann[118], Markku S Nieminen[117], Christian Hengstenberg[118], Manjinder S Sandhu[1,63], Tomi Pastinen[119], Ann-Christine Syvänen[28], G Kees Hovingh[88], George Dedoussis[51], Paul W Franks[32–34,120], Terho Lehtimäki[29,30], Andres Metspalu[18,19], Pierre A Zalloua[121], Agneta Siegbahn[12], Stefan Schreiber[94], Samuli Ripatti[1,37], Stefan S Blankenberg[74], Markus Perola[23], Robert Clarke[24,25], Bernhard O Boehm[74], Christopher O'Donnell[93], Muredach P Reilly[122,126], Winfried März[26,123], Rory Collins[24,25,127], Sekar Kathiresan[41,124,125,126], Anders Hamsten[20,21,126], Jaspal S Kooner[80,126], Unnur

Thorsteinsdottir[35,81,126], John Danesh[9, 126], Colin N A Palmer[38,126], Robert Roberts[78,79,126], Hugh Watkins[3,4,126], Heribert Schunkert[2,126] & Nilesh J Samani[31,47,126]

[1]Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK. [2]Institut für Integrative und Experimentelle Genomik, Universität zu Lübeck, Lübeck, Germany. [3]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK. [4]Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK. [5]Department of Medicine, Stanford University School of Medicine, Stanford, California, USA. [6]Department of Health Sciences, University of Leicester, Leicester, UK.[7]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden.[8]Center for Non-Communicable Diseases, Karachi, Pakistan. [9]Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK. [10]Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA. [11]Institut für Medizinische Biometrie und Statistik, Universität zu Lübeck, Lübeck, Germany. [12]Uppsala Clinical Research Center, Uppsala University, Uppsala, Sweden.[13]Division of Cardiovascular and Neuronal Remodelling, Multidisciplinary Cardiovascular Research Centre, Leeds Institute of Genetics, Health and Therapeutics, University of Leeds, Leeds, UK. [14]Center for Genome Science, Korea National Institute of Health, Korea Center for Disease Control and Prevention, Yeonje-ri, Chungwon-gun, Chungcheongbuk-do, Korea. [15]Division of Cardiovascular Medicine, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, USA.[16]Department of Human Genetics, University of Michigan, Ann Arbor, Michigan, USA. [17]Department of Epidemiology and Biostatistics, Imperial College London, London, UK. [18]Estonian Genome Center, University of Tartu, Tartu, Estonia. [19]Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia. [20]Atherosclerosis Research Unit, Department of Medicine, Karolinska Institutet, Stockholm, Sweden. [21]Center for Molecular Medicine, Karolinska University Hospital, Stockholm, Sweden.[22]Department of Twin Research and Genetic Epidemiology, King's College London, London, UK. [23]Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland. [24]Clinical Trial Service Unit, University of Oxford, Oxford, UK. [25]Epidemiological Studies Unit, University of Oxford, Oxford, UK. [26]Mannheim Institute of Public Health, Social and Preventive Medicine, Medical Faculty of Mannheim, University of Heidelberg, Mannheim, Germany. [27]Ludwigshafen Risk and Cardiovascular Health (LURIC) Study, Freiburg, Germany. [28]Department of Medical Sciences, Uppsala University, Uppsala, Sweden. [29]Department of Clinical Chemistry, Fimlab Laboratories, Tampere University Hospital, Tampere, Finland. [30]Department of Clinical Chemistry, University of Tampere School of Medicine, Tampere, Finland. [31]Department of Cardiovascular Sciences, University of Leicester, Glenfield Hospital, Leicester, UK. [32]Genetic & Molecular Epidemiology Unit, Department of Clinical Sciences, Lund University Diabetes Center, Skåne University Hospital, Malmö, Sweden. [33]Department of Public Health & Clinical Medicine, Genetic Epidemiology & Clinical Research Group, Section for Medicine, Umeå University, Umeå, Sweden. [34]Department of Odontology, Umeå University, Umeå, Sweden. [35]deCODE Genetics, Reykjavik, Iceland. [36]Institute for Molecular Medicine FIMM, University of Helsinki, Helsinki, Finland. [37]Public Health Genomics Unit, National Institute for Health and Welfare, Helsinki, Finland. [38]Medical Research Institute, University of Dundee, Ninewells

Hospital and Medical School, Dundee, UK. [39]Department of Pharmacology, University of Pennsylvania, Philadelphia, Pennsylvania, USA. [40]HudsonAlpha Institute for Biotechnology, Huntsville, Alabama, USA.[41]Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA. [42]Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts, USA. [43]Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts, USA. [44]Department of Genetics, Harvard Medical School, Boston, Massachusetts, USA. [45]Division of Cardiovascular and Diabetes Research, Multidisciplinary Cardiovascular Research Centre, Leeds Institute of Genetics, Health and Therapeutics, University of Leeds, Leeds, UK. [46]University of Cambridge Metabolic Research Laboratories, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK. [47]National Institute for Health Research (NIHR) Leicester Cardiovascular Biomedical Research Unit, Glenfield Hospital, Leicester, UK. [48] Deutsches Herzzentrum München, Technische Universität München, Munich, Germany. [49]Practice of Gynecology, Ulm University Medical Centre, Ulm, Germany. [50]Biotherapeutics and Bioinnovation Center, Pfizer, South San Francisco, California, USA. [51]Department of Dietetics– Nutrition, Harokopio University, Athens, Greece. [52]A list of members and affiliations appears in the **Supplementary Note**. [53]Klinik für Innere Medizin, Kreiskrankenhaus Rendsburg, Rendsburg, Germany. [54]Cardiothoracic Surgery Unit, Department of Molecular Medicine and Surgery, Karolinska Institutet,Stockholm, Sweden. [55]Division of Cardiovascular Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden. [56]Department of Clinical Sciences, Diabetes and Endocrinology, Lund University, University Hospital Malmö, Malmö, Sweden. [57]CEA–Genomics Institute, National Genotyping Centre, Paris, France. Commissariat à l'énergie atomique et aux energies alternatives] [58]Department of Public Health & Clinical Medicine, Section for Nutritional Research, Umeå University, Umeå, Sweden. [59]Department of Biostatistics, Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan, USA. [60]Hannover Unified Biobank, Hannover Medical School, Hannover, Germany. [61]First Cardiology Department, Onassis Cardiac Surgery Center 356, Athens, Greece. [62]Department of Medicine, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland. [63]Medical Research Council (MRC) Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK. [64]Transplantation Laboratory, Haartman Institute, University of Helsinki, Helsinki, Finland. [65]Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK. [66]Oxford NIHR Biomedical Research Centre, Churchill Hospital, Oxford, UK.[67]Institute of Epidemiology II, Helmholtz Zentrum München–German Research Center for Environmental Health, Neuherberg, Germany. [68]Institut National de la Santé et la Recherche Médicale (INSERM) Unité Mixte de Recherche (UMR) S937, Institute for Cardiometabolism and Nutrition (ICAN), Pierre and Marie Curie (Paris 6) University, Paris, France. [69]Department of Medicine I, University Hospital Grosshadern, Ludwig-Maximilians-Universität, Munich, Germany. [70]Chair of Epidemiology, Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-Universität, Munich, Germany. [71]Chair of Genetic Epidemiology, Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-Universität, Munich, Germany. [72]Institute of Genetic Epidemiology, Helmholtz Zentrum München–German Research Center for Environmental Health, Neuherberg, Germany.

[73]Heart Centre, Department of Cardiology, Tampere University Hospital, Tampere, Finland. [74]Division of Endocrinology and Diabetes, Department of Internal Medicine, Ulm University Medical Centre, Ulm, Germany. [75]Institut für Klinische Molekularbiologie, Christian-Albrechts Universität, Kiel, Germany. [76]Division of Epidemiology, Multidisciplinary Cardiovascular Research Centre (MCRC) University of Leeds, Leeds, UK. [77]Leeds Institute of Genetics, Health and Therapeutics, University of Leeds, Leeds, UK. [78]University of Ottawa Heart Institute, Cardiovascular Research Methods Centre Ontario, Ottawa, Ontario, Canada. [79]Ruddy Canadian Cardiovascular Genetics Centre, Ottawa, Ontario, Canada. [80]National Heart and Lung Institute (NHLI), Imperial College London, Hammersmith Hospital, London, UK. [81]Faculty of Medicine, University of Iceland, Reykjavik, Iceland. [82]Department of Medicine, Landspitali University Hospital, Reykjavik, Iceland. [83]Department of Experimental Immunohematology, Sanquin, Amsterdam, The Netherlands. [84]Center for Thrombosis and Hemostasis, University Medical Center Mainz, Johannes Gutenberg University Mainz, Mainz, Germany. [85]Department of Medicine 2, University Medical Center Mainz, Johannes Gutenberg University Mainz, Mainz, Germany. [86]Institut Pasteur de Lille, INSERM U744, Université Lille Nord de France, Lille, France. [87]Department of Epidemiology and Public Health, EA3430, University of Strasbourg, Strasbourg, France. [88]Department of Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands. [89]Human Genetics Center, University of Texas Health Science Center, Houston, Texas, USA. [90]Department of Experimental Medicine, University of Milano– Bicocca, Monza, Italy. [91]Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts, USA. [92]National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, Massachusetts, USA. [93]Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands. [94]Clinic for General and Interventional Cardiology, University Heart Center Hamburg, Hamburg, Germany. [95]Cardiovascular Research Institute, Washington Hospital Center, Washington, DC, USA. [96]Centre for Public Health, The Queen's University of Belfast, Belfast, UK. [97]Research Centre for Epidemiology and Preventive Medicine (EPIMED), Department of Clinical and Experimental Medicine, University of Insubria, Varese, Italy. [98]Department of Cardiology, Toulouse University School of Medicine, Rangueil Hospital, Toulouse, France. [99]INSERM UMR S872, Cordeliers Research Centre,Paris, France. [100]Division of Research, Kaiser Permanente Northern California, Oakland, California, USA. [101]Icelandic Heart Association, Kopavogur, Iceland. [102]Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio, USA. [103]Cardiology Division, Department of Internal Medicine, Cardiovascular Genome Center, Yonsei University, Seoul, Korea. [104]Department of Clinical Physiology, Tampere University Hospital and University of Tampere, Tampere, Finland. [105]UK Clinical Research Collaboration (UKCRC) Centre of Excellence for Public Health (Northern Ireland), Queen's University of Belfast, Belfast, UK. [106]Department of Internal Medicine, Cardiovascular Center, Seoul National University Hospital, Seoul, Korea. [107]Department of Internal Medicine II–Cardiology, Ulm University Medical Center, Ulm, Germany. [108]Science Center, Tampere University Hospital, Tampere, Finland. [109]Department of Haematology, University of Cambridge, Cambridge, UK. [110]National Health Service (NHS) Blood and Transplant, Cambridge, UK. [111]Division of Cardiology, Samsung Medical Center, Seoul, Korea. [112]Munich

Heart Alliance, Munich, Germany. [113]Division of Translational Medicine and Human Genetics, Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania, USA. [114]Institute for Genomics and Multiscale Biology, Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, New York, USA. [115]Center for Human Genetics, Department of Medicine, Duke University Medical Center, Durham, North Carolina, USA. [116]Division of Cardiology, Department of Medicine, Duke University Medical Center, Durham, North Carolina, USA. [117]Division of Cardiology, Department of Medicine, Helsinki University Central Hospital (HUCH), Helsinki, Finland. [118]Klinik und Poliklinik für Innere Medizin II, Regensburg, Germany. [119]Department of Human Genetics, McGill University, Montréal, Québec, Canada. [120]Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts, USA. [121]Lebanese American University, Chouran, Beirut, Lebanon. [122]Cardiovascular Institute, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania, USA. [123]Synlab Academy, Mannheim, Germany. [124]Cardiology Division, Center for Human Genetic Research, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA.[125]Cardiovascular Research Center, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA. [126]These authors contributed equally to this work.

SUPPLEMENTAL REFERENCES

(1) Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. Reporting the Results of a Meta-Analysis. *Introduction to meta-analysis*. 1st ed. John Wiley and Sons; 2009. p. 365-70.

(2) Sofat R, Hingorani AD, Smeeth L, Humphries SE, Talmud PJ, Cooper J et al. Separating the mechanism-based and off-target actions of cholesteryl ester transfer protein inhibitors with CETP gene polymorphisms. *Circulation*. 2010;121:52-62.

(3) The Lipid Research Clinics Coronary Primary Prevention Trial results. I. Reduction in incidence of coronary heart disease. *JAMA*. 1984;251:351-64.

(4) Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet*. 2005;366:1267-78.

(5) Wiklund O, Angelin B, Fager G, Eriksson M, Olofsson SO, Berglund L et al. Treatment of familial hypercholesterolaemia: a controlled trial of the effects of pravastatin or cholestyramine therapy on lipoprotein and apolipoprotein levels. *J Intern Med*. 1990;228:241-7.

(6) Betteridge DJ, Bhatnager D, Bing RF, Durrington PN, Evans GR, Flax H et al. Treatment of familial hypercholesterolaemia. United Kingdom lipid clinics study of pravastatin and cholestyramine. *BMJ*. 1992;304:1335-8.

(7) Brensike JF, Levy RI, Kelsey SF, Passamani ER, Richardson JM, Loh IK et al. Effects of therapy with cholestyramine on progression of coronary arteriosclerosis: results of the NHLBI Type II Coronary Intervention Study. *Circulation*. 1984;69:313-24.

(8) Comparative efficacy and safety of pravastatin and cholestyramine alone and combined in patients with hypercholesterolemia. Pravastatin Multicenter Study Group II. *Arch Intern Med*. 1993;153:1321-9.

(9) Watts GF, Lewis B, Brunt JN, Lewis ES, Coltart DJ, Smith LD et al. Effects on coronary artery disease of lipid-lowering diet, or diet plus cholestyramine, in the St Thomas' Atherosclerosis Regression Study (STARS). *Lancet*. 1992;339:563-9.

(10) Hunninghake DB, Stein EA, Bremner WF, Greenland P, Demke DM, Oliphant TH. Dose--Response Study of Colestipol Tablets in Patients with Moderate Hypercholesterolemia. *Am J Ther*. 1995;2:180-9.

(11) Simons LA, Simons J, Parfitt A. Successful management of primary hypercholesterolaemia with simvastatin and low-dose colestipol. *Med J Aust*. 1992;157:455-9.

(12) Superko HR, Greenland P, Manchester RA, Andreadis NA, Schectman G, West NH et al. Effectiveness of low-dose colestipol therapy in patients with moderate hypercholesterolemia. *Am J Cardiol*. 1992;70:135-40.

(13) Insull W, Jr., Toth P, Mullican W, Hunninghake D, Burke S, Donovan JM et al. Effectiveness of colesevelam hydrochloride in decreasing LDL cholesterol in patients with primary hypercholesterolemia: a 24-week randomized controlled trial. *Mayo Clin Proc*. 2001;76:971-82.

(14) Bays HE, Goldberg RB, Truitt KE, Jones MR. Colesevelam hydrochloride therapy in patients with type 2 diabetes mellitus treated with metformin: glucose and lipid effects. *Arch Intern Med*. 2008;168:1975-83.

(15) Davidson MH, Dillon MA, Gordon B, Jones P, Samuels J, Weiss S et al. Colesevelam hydrochloride (cholestagel): a new, potent bile acid sequestrant associated with a low incidence of gastrointestinal side effects. *Arch Intern Med*. 1999;159:1893-900.

(16) Devaraj S, Autret B, Jialal I. Effects of colesevelam hydrochloride (WelChol) on biomarkers of inflammation in patients with mild hypercholesterolemia. *Am J Cardiol*. 2006;98:641-3.

(17) Handelsman Y, Goldberg RB, Garvey WT, Fonseca VA, Rosenstock J, Jones MR et al. Colesevelam hydrochloride to treat hypercholesterolemia and improve glycemia in prediabetes: a randomized, prospective study. *Endocr Pract*. 2010;16:617-28.

(18) Hunninghake D, Insull W, Jr., Toth P, Davidson D, Donovan JM, Burke SK. Coadministration of colesevelam hydrochloride with atorvastatin lowers LDL cholesterol additively. *Atherosclerosis*. 2001;158:407-16.

(19) Knapp HH, Schrott H, Ma P, Knopp R, Chin B, Gaziano JM et al. Efficacy and safety of combination simvastatin and colesevelam in patients with primary hypercholesterolemia. *Am J Med*. 2001;110:352-60.

(20) Rosenstock J, Fonseca VA, Garvey WT, Goldberg RB, Handelsman Y, Abby SL et al. Initial combination therapy with metformin and colesevelam for achievement of glycemic and lipid goals in early type 2 diabetes. *Endocr Pract*. 2010;16:629-40.

(21) Zieve FJ, Kalin MF, Schwartz SL, Jones MR, Bailey WL. Results of the glucose-lowering effect of WelChol study (GLOWS): a randomized, double-blind, placebo-controlled pilot study evaluating the effect of colesevelam hydrochloride on glycemic control in subjects with type 2 diabetes. *Clin Ther*. 2007;29:74-83.

Circulation
Cardiovascular Genetics

Effect of Bile Acid Sequestrants on the Risk of Cardiovascular Events: A Mendelian Randomization Analysis

Stephanie Ross, Matthew D'Mello, Sonia S. Anand, John Eikelboom, CARDIoGRAMplusC4D Consortium, Alexandre F.R. Stewart, Nilesh J. Samani, Robert Roberts and Guillaume Paré

Circ Cardiovasc Genet. 2015;8:618-627; originally published online June 4, 2015;
doi: 10.1161/CIRCGENETICS.114.000952
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1942-325X. Online ISSN: 1942-3268