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 coleselam. 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 coleselam 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=6.3×10⁻⁵) and coleselam 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.

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). Current guidelines recommend that statin therapy should be used in select groups of patients with atherosclerotic CVD in primary and secondary prevention settings. However, statins may not be fully effective in lowering LDL-C or well tolerated, 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. Three BAS have been approved for clinical use: cholestyramine and colestipol (first generation) and coleselam hydrochloride (coleselam; second generation). Coleselam was developed to overcome gastrointestinal intolerance associated with the first-generation BAS. Three randomized controlled trials (RCTs) have evaluated the efficacy of cholestyramine for cardiovascular prevention, but results have been inconclusive.

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.), 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.).

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

Circ Cardiovasc Genet is available at http://circgenetics.ahajournals.org

DOI: 10.1161/CIRCGENETICS.114.000952

618
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; \( P = 0.07 \)).\(^6\) 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.\(^6\) 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 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 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 92,851 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.\(^{24}\) 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).\(^3\) 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.\(^7\)
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=39612) and 21 trials that compared statin to a control (N=129526). 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 Supplement and Table I in the Data Supplement.

Randomized Controlled Trials of Colesevelam
We identified a total of 6 RCTs comprising 4598 hyperlipidemia patients,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). 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 pooled estimates owing to a lack of reported data from clinical trials, the results of the colestipol meta-analysis are described in Methods in the Data Supplement and Table I in the Data Supplement.

Randomized Controlled Trials of Cholestyramine
We identified 10 trials with a total of 1142 participants with hyperlipidemia and 883 participants with type 2 diabetes mellitus.20–29 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 (F 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.

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.
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. 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 mg/dL per allele (95% CI = −3.44, −2.58) mg/dL per allele (\( P=4.0\times10^{-45} \)), a decrease in...
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 (HbA1c), fasting glucose, systolic blood pressure, diastolic blood pressure, and body mass index using data from the DIAGRAM,38 MAGIC,39,40 GIANT,41 and ICBP42 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 trial8 (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 \(OR=0.64 (95\% \text{ CI } 0.52–0.79; P=4.3\times10^{-5})\) 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 \((OR 0.86, 95\% \text{ CI } 0.85–0.87; P=6.6\times10^{-8})\). The change in LDL-C levels from 24 g/d colesevelam was predicted to significantly decrease the risk of major vascular events \((OR 0.86, 95\% \text{ CI } 0.85–0.87; P=6.6\times10^{-8})\). The change in LDL-C levels from 24 g/d colesevelam was predicted to significantly decrease the risk of major vascular events \((OR 0.86, 95\% \text{ CI } 0.85–0.87; P=6.6\times10^{-8})\). 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
to significantly reduce the risk of cardiovascular events (OR 0.90, 95% CI 0.87–0.93; \( P = 1.3 \times 10^{-13} \); \( P \) 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
trials have failed to show a benefit of Niacin and CETP inhibitors.\textsuperscript{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.\textsuperscript{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.\textsuperscript{5} Colesevelam is much better tolerated,\textsuperscript{46,47} has other potential benefits, such as reducing fasting blood glucose levels,\textsuperscript{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.\textsuperscript{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.\textsuperscript{50} Thus, our results suggest a beneficial effect of colesevelam on risk of CAD and highlight the need for well-designed RCTs.

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.

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 3.75 g/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.
to fully understand the clinical efficacy and safety of colesvelam 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. 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. Overexpression of ABCG5/8 genes in transgenic mice resulted in an increase in biliary cholesterol secretion, reduced cholesterol absorption, and increased hepatic cholesterol synthesis, 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. Although the underlying mechanism is unknown, it has been suggested that the binding of BAS to bile acids alters the GI tract glucose absorption. 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. In our study, we did not observe an association of rs4299376 SNP with the changes in the levels of fasting glucose or HbA\textsubscript{1c} and diabetes mellitus using data from the MAGIC and DIAGRAM Consortia (P>0.05 for all), 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 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, 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. 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 colesvelam 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. 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/css/abcassets/public/lipids2010/.

Sources of Funding

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

Disclosures

None.

References


randomization in drug target validation and to complement clinical trial data. This study also highlights the use of Mendelian randomization in drug target validation and clinical trials to assess the effect of BAS on plasma lipid levels and CAD outcomes. We then applied the principles of Mendelian randomization in drug target validation and to complement clinical trial data.

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 to statins for whom statins are ineffective in reducing low-density lipoprotein cholesterol. This study also highlights the use of Mendelian randomization in drug target validation and clinical trials to assess the effect of BAS on plasma lipid levels and CAD outcomes. We then applied the principles of Mendelian randomization in drug target validation and to complement clinical trial data.
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
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 1942-325X. Online ISSN: 1942-3268

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circgenetics.ahajournals.org/content/8/4/618

Data Supplement (unedited) at:
http://circgenetics.ahajournals.org/content/suppl/2015/06/04/CIRCGENETICS.114.000952.DC1

**Permissions**: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation: Cardiovascular Genetics_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

**Reprints**: Information about reprints can be found online at:
http://www.lww.com/reprints

**Subscriptions**: Information about subscribing to _Circulation: Cardiovascular Genetics_ is online at:
http://circgenetics.ahajournals.org/subscriptions/
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 colesvelam) 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; colesvelam 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 ($\chi^2$) 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\textsuperscript{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\textsuperscript{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\textsuperscript{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\(^3\)\(^{-9}\), three colestipol RCTs\(^{10-12}\) and 10 colesvelam RCTs\(^{13-21}\) with a total of 7,021 study participants. Supplemental Figure 1 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 colesvelam 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\textsuperscript{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 $=1.70\times10^{-4}$) while an $r=0.7$ significantly increased the presence of heterogeneity (heterogeneity $P$-value:$2.10\times10^{-9}$). Similar results were also obtained for the treatment effects of colesevelam.
Supplemental Table #1: Studies contributing to the colestipol meta-analysis

<table>
<thead>
<tr>
<th>Author &amp; Date</th>
<th>Patient Population</th>
<th>Sample size</th>
<th>Intervention</th>
<th>Comparison</th>
<th>Follow-Up</th>
<th>Age (Mean, SD)</th>
<th>Women (%)</th>
<th>European (%)</th>
<th>LDL-C (mg/dL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunninghake 1995&lt;sup&gt;10&lt;/sup&gt;</td>
<td>Hyperlipidemia</td>
<td>196</td>
<td>Colestipol (2 g; 4 g; 8 g; 16 g)</td>
<td>Placebo</td>
<td>8 weeks</td>
<td>56.2 (NR)</td>
<td>101 (52)</td>
<td>NR</td>
<td>190.0(NR)</td>
</tr>
<tr>
<td>Simons 1992&lt;sup&gt;11&lt;/sup&gt;</td>
<td>Hyperlipidemia</td>
<td>61</td>
<td>Colestipol (5 g); Colestipol (10 g) &amp; each with 6 weeks of placebo; 6 weeks of simvasatin (20 mg); 6 weeks of simvasatin (40 mg)</td>
<td>Placebo with 6 weeks of placebo; 6 weeks of simvasatin (20 mg); 6 weeks of simvasatin (40 mg)</td>
<td>18 weeks</td>
<td>45.3 (19)</td>
<td>24 (39)</td>
<td>26 (43)</td>
<td>303.1(77.7)</td>
</tr>
<tr>
<td>Superko 1992&lt;sup&gt;12&lt;/sup&gt;</td>
<td>Hyperlipidemia</td>
<td>141</td>
<td>Colestipol (5g/d; 10g/d; 15g/d)</td>
<td>Placebo</td>
<td>12 weeks</td>
<td>49(12)</td>
<td>49 (35)</td>
<td>NR</td>
<td>168.0(12.0)</td>
</tr>
</tbody>
</table>

*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 (HbA1c), fasting glucose (FG), systolic blood pressure (SBP), diastolic blood pressure (DBP) and body mass index (BMI).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Effect Allele</th>
<th>Other Allele</th>
<th>Effect Estimate</th>
<th>Standard Error</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C</td>
<td>T</td>
<td>G</td>
<td>-2.75</td>
<td>0.19898</td>
<td>1.73E-47</td>
</tr>
<tr>
<td>HDL-C</td>
<td>T</td>
<td>G</td>
<td>0.05</td>
<td>0.07143</td>
<td>0.212</td>
</tr>
<tr>
<td>TC</td>
<td>T</td>
<td>G</td>
<td>-3.01</td>
<td>0.21939</td>
<td>4.00E-45</td>
</tr>
<tr>
<td>TG</td>
<td>T</td>
<td>G</td>
<td>-1.08</td>
<td>0.36735</td>
<td>0.003</td>
</tr>
<tr>
<td>FG</td>
<td>G</td>
<td>T</td>
<td>0.00088</td>
<td>0.0026</td>
<td>0.737689</td>
</tr>
<tr>
<td>HbA1c</td>
<td>T</td>
<td>G</td>
<td>-0.0051</td>
<td>0.004</td>
<td>0.199</td>
</tr>
<tr>
<td>Diabetes</td>
<td>G</td>
<td>T</td>
<td>-0.00738</td>
<td>0.016336</td>
<td>0.65164</td>
</tr>
<tr>
<td>SBP</td>
<td>G</td>
<td>T</td>
<td>0.024683</td>
<td>0.112445</td>
<td>0.826253</td>
</tr>
<tr>
<td>DBP</td>
<td>G</td>
<td>T</td>
<td>0.00435</td>
<td>0.070956</td>
<td>0.951115</td>
</tr>
<tr>
<td>BMI</td>
<td>T</td>
<td>G</td>
<td>-0.0054</td>
<td>0.0064</td>
<td>0.4</td>
</tr>
</tbody>
</table>
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.

Records identified through database searching (n = 420)

Additional records identified through other sources (n = 0)

Records after duplicates removed (n = 0)

Records screened (n = 420)

Records excluded (n = 360)
- Duplicate publications
- Trial design
- Study intervention
- Patient population
- Outcome not measured

Full-text articles assessed for eligibility (n = 60)

Full-text articles excluded (n = 41)
- Duplicate publications: 6
- Trial design: 27
- Study intervention: 8

Studies included in qualitative synthesis (n = 19)

Studies included in quantitative synthesis (meta-analysis) (n = 10)
List of CARDIoGRAMplusC4D authors

Panos Deloukas¹,126, Stavroula Kanoni¹,126, Christina Willenborg²,126, Martin Farrall³,4,126, Themistocles L. Assimes⁵,126, John R Thompson⁶,126, Erik Ingelsson⁷,126, Danish Saleheen⁷,10,126, Jeanette Erdmann²,126, Benjamin A Goldstein⁵, Kathleen Stirrups¹, Inke R König¹, Jean-Baptiste Cazier⁴, Åsa Johansson¹², Alistair S Hall¹³, Jong-Young Lee¹⁴, Cristen J Willer¹⁵,16, John C Chambers¹⁷, Tônú Esko¹⁸,19, Lasse Folkersen²⁰,2¹, Anju Goel³,4, Elin Grundberg²², Aki S Havulinna²³, Weang K Ho¹⁰, Jemma C Hopewell²⁴,2⁵, Niclas Eriksson¹², Marcus E Kleber²⁶,2⁷, Kati Kristiansson²³, Per Lundmark²⁸, Leo-Pekka Lyytikäinen²⁹,3⁰, Suzanne Rafelt³¹, Dmitry Shungin³²-³³, Rona J Strawbridge²⁰,2¹, Gudmar Thorleifsson³⁵, Emmi Tikkanen³⁶,³⁷, Natalie Van Zuydam³⁸, Benjamin F Voight³⁹, Lindsay L Waite⁴⁰, Weihua Zhang¹⁷, Andreas Ziegler¹¹, Devin Absher⁴⁰, David Altshuler⁴¹-⁴⁴, Anthony J Balmforth⁴⁵, Inês Barroso¹,4⁶, Peter S Braund³¹,³⁷, Christof Burgdorf⁴⁸, Simone Claudi-Boehm⁴⁹, David Cox⁵⁰, Maria Dimitriou⁵¹, Ron Do³¹,4³, CARDIOGENICS Consortium⁵², DIAGRAM Consortium⁵², Alex S F Doney³⁸, NourEddine El Mokhtari³⁵, Per Eriksson²⁰,²¹, Krista Fischer¹⁸, Pierre Fontanillas⁴¹, Anders Franco-Cereceda⁵⁴, Bruna Gigante⁵⁵, Leif Groop⁵⁶, Stefan Gustafsson⁷, Jörg Hager⁵⁷, Göran Hallmans³⁸,³⁹, Bok-Ghee Han¹⁴, Sarah E Hunt¹, Hyun M Kang⁵⁹, Thomas Illig⁶⁰, Thorsten Kessler⁴⁸, Joshua W Knowles⁵, Genovefa Kolovou⁶¹, Johanna Kuusisto⁶², Claudia Langenberg⁶³, Cordelia Langford¹, Karin Leander⁵⁵, Marja-Liisa Lokki⁶⁴, Anders Lundmark²⁸, Mark I McCarthy³,⁶⁵,⁶⁶, Christa Meisinger⁶⁷, Olle Melander⁵⁶, Evelin Mihailov¹⁹, Seiya Maouche⁶⁸, Andrew D Morris³⁸, Martina Müller-Nurasyid⁶⁹-⁷², MuTHER Consortium⁵², Kjell Nikus⁷³, John F Peden³, N William Rayner³, Asif Rasheed⁹, Silke Rosinger⁷⁴, Diana Rubin⁵³, Moritz P Rumpf⁴⁸, Arne Schäfer⁷⁵, Mohan Sivananthan⁷⁶,⁷⁷, Ci Song⁷, Alexandre F R Stewart⁷⁸,⁷⁹, Sian-Tsang Tan⁸⁰, Gudmundur Thorgerirsson⁸¹,⁸², C Ellen van der Schoot⁸³, Peter J Wagner³⁶,³⁷, Wellcome Trust Case Control Consortium⁵², George A Wells⁷⁸,⁷⁹, Philipp S Wild³⁴,⁸⁵, Tsun-Po Yang¹, Philippe Amouyel⁸⁶, Dominique Arveiller⁸⁷, Hanneke Basart⁸⁸, Michael Boehnke⁵⁹, Eric Boerwinkle⁸⁹, Paolo Brambilla⁹⁰, François Cambien⁶⁸, Adrienne L Cupples⁹¹,⁹², Ulf de Faire⁵⁵, Abbas Dehghan⁹³, Patrick Diemert⁹⁴, Stephen E Epstein⁹⁵, Alun Evans⁹⁶, Marco M Ferrarío⁹⁷, Jean Ferrières⁹⁸, Dominique Gaugier⁵,⁹⁹, Alan S Go¹⁰⁰, Alison H Goodall³¹,⁴⁷, Villi Gudnason⁸¹,¹⁰⁰, Stanley L Hazen¹⁰², Hilma Holm³⁵, Carlos Iribarren¹⁰⁰, Yangsoo Jang¹⁰³, Mika Kähönen¹⁰⁴, Frank Kee¹⁰⁵, Hyo-Soo Kim¹⁰⁶, Norman Klop⁶⁰, Wolfgang Koenig¹⁰⁷, Wolfgang Kratzer¹⁰⁸, Kari Kuulasmaa²³, Markku Laakso⁶², Reijo Laaksonen¹⁰⁸, Ji-Young Lee¹⁴, Lars Lind²⁸, Willem H Ouwehand¹,¹⁰⁹,¹¹⁰, Sarah Parish²⁴,²⁵, Jeong E Park¹¹¹, Nancy L Pedersen⁷, Annette Peters⁶⁷,¹², Thomas Quertermous⁵, Daniel J Rader¹¹³, Veikko Salomaa²³, Eric Schadt¹¹⁴, Svati H Shah¹¹⁵,¹¹⁶, Juha Sinisalo¹¹⁷, Klaus Stark¹¹⁸, Kari Stefansson³⁵,³⁸, David- Alexandre Trégouët⁶⁸, Jarmo Virtamo²³, Lars Wallentin¹², Nicholas Wareham⁶³, Martina E Zimmermann¹¹⁸, Markku S Nieminen¹¹⁷, Christian Hengstenberg¹¹⁸, Manjinder S Sandhu¹,⁶³, Tomi Pastinen¹¹⁹, Anne-Christine Syvänen²⁸, G Kees Hovingh⁸⁸, George Dedoussis⁵¹, Paul W Franks³²-³⁴,³¹², Terho Lehtimäki²⁹,³⁰, Andres Metspalu¹⁸,¹⁹, Pierre A Zalloua¹²¹, Agneta Siegbahn¹², Stefan Schreiber⁹⁴, Samuli Ripatti¹,³⁷, Stefan S Blankenberg⁷⁴, Markus Perola²³, Robert Clarke²⁴,²⁵, Bernhard O Boehm⁷⁴, Christopher O’Donnell⁹³, Muredach P Reilly¹²²,¹²⁶, Winfried März²⁶,¹²³, Rory Collins²⁴,²⁵,¹²⁷, Sekar Kathiresan⁴¹,¹²⁴,¹²⁵,¹²⁶, Anders Hamsten²⁰,²¹,¹²⁶, Jaspal S Kooner⁸⁰,¹²⁶, Unnur

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, Yeonge-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.  

39Department of Pharmacology, University of Pennsylvania, Philadelphia, Pennsylvania, USA.  

40HudsonAlpha Institute for Biotechnology, Huntsville, Alabama, USA.  

41Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA.  

42Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts, USA.  

43Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts, USA.  

44Department of Genetics, Harvard Medical School, Boston, Massachusetts, USA.  

45Division of Cardiovascular and Diabetes Research, Multidisciplinary Cardiovascular Research Centre, Leeds Institute of Genetics, Health and Therapeutics, University of Leeds, Leeds, UK.  

46University of Cambridge Metabolic Research Laboratories, Institute of Metabolic Science, Addenbrooke’s Hospital, Cambridge, UK.  

47National Institute for Health Research (NIHR) Leicester Cardiovascular Biomedical Research Unit, Glenfield Hospital, Leicester, UK.  

48Deutsches Herzzentrum München, Technische Universität München, Munich, Germany.  

49Practice of Gynecology, Ulm University Medical Centre, Ulm, Germany.  

50Biotherapeutics and Bioinnovation Center, Pfizer, South San Francisco, California, USA.  

51Department of Dietetics– Nutrition, Harokopio University, Athens, Greece.  

52A list of members and affiliations appears in the Supplementary Note.  

53Klinik für Innere Medizin, Kreiskrankenhaus Rendsburg, Rendsburg, Germany.  

54Cardiothoracic Surgery Unit, Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden.  

55Division of Cardiovascular Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.  

56Department of Clinical Sciences, Diabetes and Endocrinology, Lund University, University Hospital Malmö, Malmö, Sweden.  

57CEA–Genomics Institute, National Genotyping Centre, Paris, France. Commissariat à l’énergie atomique et aux energies alternatives]  

58Department of Public Health & Clinical Medicine, Section for Nutritional Research, Umeå University, Umeå, Sweden.  

59Department of Biostatistics, Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan, USA.  

60Hannover Unified Biobank, Hannover Medical School, Hannover, Germany.  

61First Cardiology Department, Onassis Cardiac Surgery Center 356, Athens, Greece.  

62Department of Medicine, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland.  

63Medical Research Council (MRC) Epidemiology Unit, Institute of Metabolic Science, Addenbrooke’s Hospital, Cambridge, UK.  

64Transplantation Laboratory, Haartman Institute, University of Helsinki, Helsinki, Finland.  


66Oxford NIHR Biomedical Research Centre, Churchill Hospital, Oxford, Oxford, UK.  

67Institute of Epidemiology II, Helmholtz Zentrum München–German Research Center for Environmental Health, Neuherberg, Germany.  

68Institut 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.  

69Department of Medicine I, University Hospital Grosshadern, Ludwig-Maximilians-Universität, Munich, Germany.  

70Chair of Epidemiology, Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-Universität, Munich, Germany.  

71Chair of Genetic Epidemiology, Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-Universität, Munich, Germany.  

72Institute of Genetic Epidemiology, Helmholtz Zentrum München–German Research Center for Environmental Health, Neuherberg, Germany.
Heart Centre, Department of Cardiology, Tampere University Hospital, Tampere, Finland.  
Division of Endocrinology and Diabetes, Department of Internal Medicine, Ulm University Medical Centre, Ulm, Germany.  
Institut für Klinische Molekularbiologie, Christian-Albrechts Universität, Kiel, Germany.  
Division of Epidemiology, Multidisciplinary Cardiovascular Research Centre (MCRC) University of Leeds, Leeds, UK.  
Leeds Institute of Genetics, Health and Therapeutics, University of Leeds, Leeds, UK.  
University of Ottawa Heart Institute, Cardiovascular Research Methods Centre Ontario, Ottawa, Ontario, Canada.  
Ruddy Canadian Cardiovascular Genetics Centre, Ottawa, Ontario, Canada.  
National Heart and Lung Institute (NHLI), Imperial College London, Hammersmith Hospital, London, UK.  
Faculty of Medicine, University of Iceland, Reykjavik, Iceland.  
Department of Medicine, Landspitali University Hospital, Reykjavik, Iceland.  
Department of Experimental Immunohematology, Sanquin, Amsterdam, The Netherlands.  
Center for Thrombosis and Hemostasis, University Medical Center Mainz, Johannes Gutenberg University Mainz, Mainz, Germany.  
Department of Medicine 2, University Medical Center Mainz, Johannes Gutenberg University Mainz, Mainz, Germany.  
Institut Pasteur de Lille, INSERM U744, Université Lille Nord de France, Lille, France.  
Department of Epidemiology and Public Health, EA3430, University of Strasbourg, Strasbourg, France.  
Department of Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands.  
Human Genetics Center, University of Texas Health Science Center, Houston, Texas, USA.  
Department of Experimental Medicine, University of Milano–Bicocca, Monza, Italy.  
Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts, USA.  
National Heart, Lung, and Blood Institute’s Framingham Heart Study, Framingham, Massachusetts, USA.  
Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands.  
Clinic for General and Interventional Cardiology, University Heart Center Hamburg, Hamburg, Germany.  
Cardiovascular Research Institute, Washington Hospital Center, Washington, DC, USA.  
Centre for Public Health, The Queen’s University of Belfast, Belfast, UK.  
Research Centre for Epidemiology and Preventive Medicine (EPIMED), Department of Clinical and Experimental Medicine, University of Insubria, Varese, Italy.  
Department of Cardiology, Toulouse University School of Medicine, Rangueil Hospital, Toulouse, France.  
INSERM UMR S872, Cordeliers Research Centre, Paris, France.  
Division of Research, Kaiser Permanente Northern California, Oakland, California, USA.  
Icelandic Heart Association, Kopavogur, Iceland.  
Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio, USA.  
Cardiology Division, Department of Internal Medicine, Cardiovascular Genome Center, Yonsei University, Seoul, Korea.  
Department of Clinical Physiology, Tampere University Hospital and University of Tampere, Tampere, Finland.  
UK Clinical Research Collaboration (UKCRC) Centre of Excellence for Public Health (Northern Ireland), Queen’s University of Belfast, Belfast, UK.  
Department of Internal Medicine, Cardiovascular Center, Seoul National University Hospital, Seoul, Korea.  
Department of Internal Medicine II–Cardiology, Ulm University Medical Center, Ulm, Germany.  
Science Center, Tampere University Hospital, Tampere, Finland.  
Department of Haematology, University of Cambridge, Cambridge, UK.  
National Health Service (NHS) Blood and Transplant, Cambridge, UK.  
Division of Cardiology, Samsung Medical Center, Seoul, Korea.  
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**


