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Mendelian randomization studies on atherosclerotic cardiovascular disease: evidence and limitations

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Epidemiological research has revealed a galaxy of biomarkers, such as genes, molecules or traits, which are associated with increased risk of atherosclerotic cardiovascular diseases (ASCVD). However, the etiological basis remains poorly characterized. Mendelian randomization (MR) involves the use of observational genetic data to ascertain the roles of disease-associated risk factors and, in particular, differentiate those reflecting the presence or severity of a disease from those contributing causally to a disease. Over the past decade, MR has evolved into a fruitful approach to clarifying the causal relation of a biomarker with ASCVD and to verifying potential therapeutic targets for ASCVD. In this review, we selected high-quality MR studies on ASCVD, examined the causal relationship of a series of biomarkers with ASCVD, and elucidated the role of MR in validating biomarkers as a therapeutic target by comparing the results from MR studies and randomized clinical trials (RCTs) for the treatment of ASCVD. The good agreement between the results derived by MR and RCTs suggests that MR could be performed as a screening process before novel drug development. However, when designing and interpreting a MR study, the assumptions and limitations inherent in this approach should be taken into account. Novel methodological developments, such as sensitivity analysis, will help to strengthen the validity of MR studies.

Mendelian randomization, atherosclerotic cardiovascular disease, causality, therapeutic target

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Introduction

Randomized controlled trials (RCTs) are uniformly considered the ideal means to provide robust evidence as to whether certain exposures are causal factors for diseases of public health interest. In situations where the presence of exposures is not practical or ethical for randomization in humans, observational studies represent an alternative approach to searching for evidence. However, observed associations are often proven spurious by RCTs due to their inherent limitations including confounding, reverse causation and biases (Vandenbroucke, 2004).

To overcome the limitations of observational design, genetic variants have been proposed as potential instrumental variables (IVs) to simulate the effects of modifiable environmental exposures on the susceptibility of a disease, referred to as Mendelian randomization (MR) (Bochud and Rousson, 2010). An important feature of genetic differences is that they are randomly assorted according to the Mendelian second law and unchanged during the human lifetime,

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and thus are not associated with known or unknown confounders (Davey Smith and Hemani, 2014). In standard MR, the basic principles involve three assumptions (Figure S1 in Supporting Information): (1) a genetic variant is associated with an exposure or intermediate phenotype (IP) and the former may influence the propensity, affect the level, or imitate the biological effects of the latter, on the basis of known knowledge; (2) the genetic variant is not associated with the outcome except through the exposure or IP; and (3) the genetic variant is unrelated with known or unknown confounders. MR offers the following advantages over observational epidemiology. First, MR design is not prone to reverse causation because a genotype is determined at conception. Second, MR studies are relatively immune to common behavioral, physiological and socioeconomic confounders because of random assignment of alleles at meiosis. Third, genetic variants can be, in most cases, precisely measured and reported, thus not subject to biases and errors, which is especially useful in evaluating a risk factor of long-term effects. Therefore, MR, resembling an RCT, can be used as an effective tool to estimate the causality with observational data, with much less concerns of ethical, applicability and financial issues. The major difference between MR studies and RCTs is that in most RCTs, participants are randomly allocated to receive one or other of the alternative treatments, whereas in MR studies, the random allocation is based on carriage of a certain effect allele or not.

Applications of MR for atherosclerotic cardiovascular disease

Atherosclerotic cardiovascular diseases (ASCVD) comprise a broad spectrum of clinical entities and we will focus on MR studies on subclinical AS and coronary artery disease (CAD) in this review. Epidemiological research over the last 50 years has revealed a wealth of biomarkers associated with increased risk of ASCVD; however, the causality remains largely undefined. Several genome-wide association studies (GWASs) of ASCVD have recently identified a plethora of common variants at a number of genomic loci (Björkegren et al., 2015).

In light of the advances in MR studies in ASCVD, several reviews have appeared in recent literature to introduce the latest developments (Jansen et al., 2014; Mokry et al., 2015). However, these reviews paid little attention to the quality measure and methodological validity of published MR studies, such as instrumental variable selection, population stratification, confounding adjustment, pleiotropy control and power estimation. In addition, a group of novel biomarkers has not been reviewed yet. Moreover, new methodological developments in MR studies have challenged previously recognized causality of certain biomarkers. Here we have provided an explicit overview of the published evidence of MR studies on ASCVD by paying a great attention to their limitations and designing strict literature search strategy and selection criteria.

Search strategy and selection criteria

Relevant original studies were identified by searching for articles published from January 2003 in MEDLINE (Ovid), EMBASE, the Cochrane Library (Cochrane Central Register of Controlled Trials), and Grey Literature database. We also considered published review articles and editorials. The search algorithm for MEDLINE was as follows: 'Mendelian randomisation' or 'Mendelian randomization' or 'genetic instrumental variable' or a related term (e.g. 'genetic instrument') and 'atherosclerotic cardiovascular disease' or 'atherosclerosis' or 'angina' or 'coronary heart disease' or 'coronary artery disease (CAD)' or 'myocardial infarction', with no restriction on subheadings. All retrieved articles were checked for relevant citations and studies not included in the above electronic sources were searched manually. We included reports of clinical studies with the following criteria: (1) studies used MR methodology and instrumental variable analysis to evaluate risk factors of ASCVD; (2) studies were performed on the basis of prospective or cross-sectional study design; (3) the sample size in each study was ≥5,000. Finally, 58 studies were included and reviewed.

Causality between serum lipid levels and ASCVD

Low-density lipoprotein cholesterol

In recent years, a number of MR studies have firmly established the causal relationship between low-density lipoprotein cholesterol (LDL-C) and the risk of CAD (Figure 1). A MR analysis using LDL-C genetic risk scores (GRS) based on single nucleotide polymorphisms (SNPs) specific for high-density lipoprotein cholesterol (HDL-C), LDL-C and triglycerides (TG) confirmed a causal relationship between LDL-C level and carotid intima-media thickness (CIMT) as a subclinical measure of AS, but not between HDL-C and TG levels and CIMT (Shah et al., 2013).

In several studies (Linsel-Nitschke et al., 2008; Ference et al., 2016), conclusions from MR studies were consistent with the results of LDL-C lowering RCTs. From a clinical viewpoint, the reduction of LDL-C level by genotype variant appears to be very small, yet the risk reduction for CAD events was almost as much as that obtained by statin treatment (Grundy et al., 2004), possibly due to the beneficial effects of lifelong LDL-C lowering by genetic variants (Linsel-Nitschke et al., 2008).

Ezetimibe is an inhibitor of cholesterol absorption in the

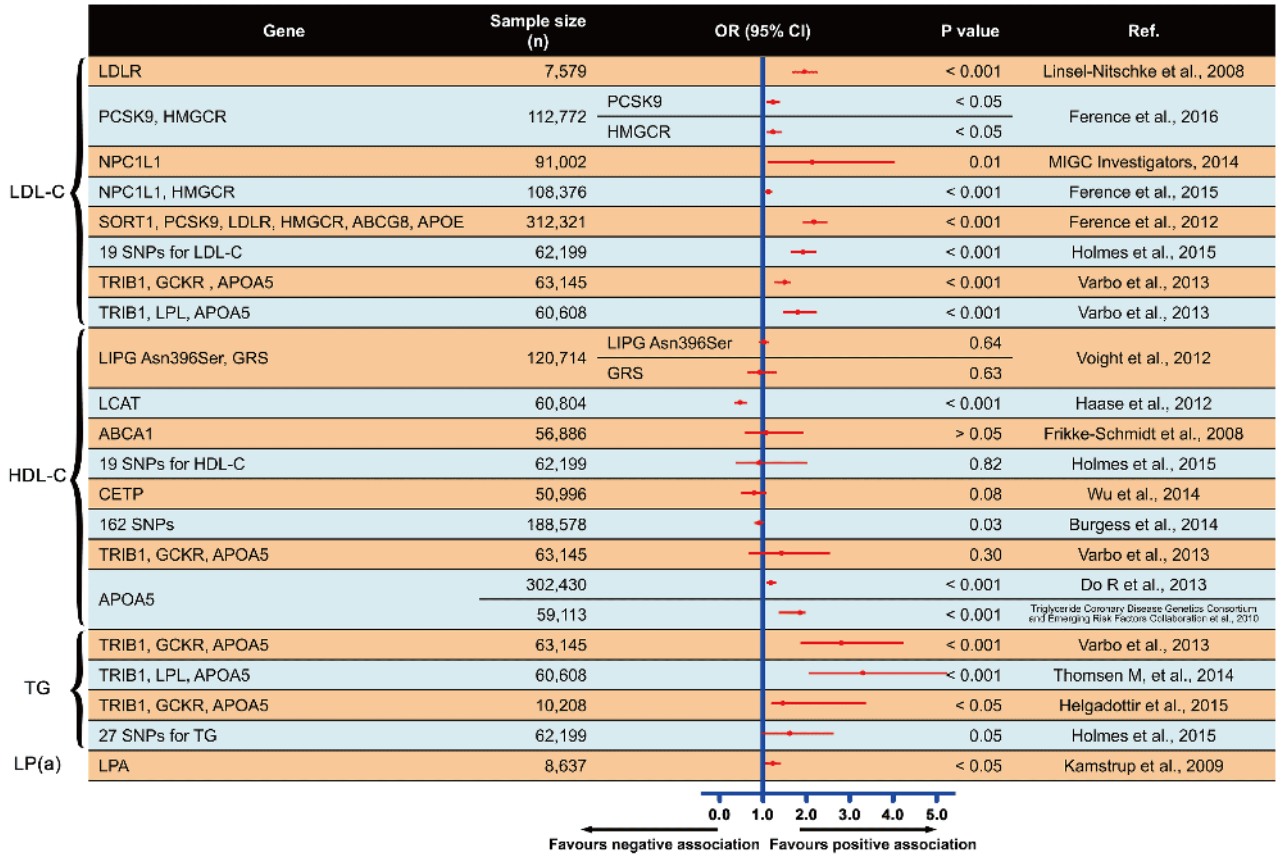

Figure 1 (Color online) Relation between serum lipids and coronary artery disease in Mendelian randomization studies.

small intestine. As the mechanism of serum levels of LDL-C lowering by Ezetimibe involves inhibition of the activity of the Niemann-Pick C1-like 1 (NPC1L1) protein, three MR analyses have identified NPC1L1 gene variation being associated with serum LDL-C levels and CAD risk (Polisecki et al., 2010; Myocardial Infarction Genetics Consortium Investigators, 2014; Ference et al., 2015). Of note, two MR studies using 2×2 factorial design indicated that the effect of LDL-C lowering on the risk of CAD is mainly due to the absolute reduction of LDL-C levels rather than the therapeutic mechanisms by which LDL-C is lowered (Ference et al., 2015, 2016). Regardless of negative result of the ENHANCE trial (Kastelein et al., 2008), the IMPROVE-IT showed that in comparison with simvastatin treatment alone, the combination of ezetimibe and simvastatin treatment resulted in additional reduction of serum LDL-C levels and cardiovascular events (Cannon et al., 2015). Thus, ezetimibe was finally rescued by CRT using hard-end points and MR studies.

Another 2×2 factorial MR analysis on proprotein convertase subtilisin/kexin type 9 (PCSK9) gene also indicated a dose-dependent log-linear association between PCSK9-mediated lower LDL-C levels and the risk of MI or death from CAD (Ference et al., 2016). A monoclonal PCSK9 antibody has also been proven effective in remarkably reducing cardiovascular events (Sabatine et al., 2015; Sabatine et al., 2017). Therefore, ACC experts have recently recommended non-statin therapies for LDL-C lowering in the management of ASCVD (Lloyd-Jones et al., 2016). Based on MR studies, we cannot get a conclusion that PCSK9 inhibitors would be more effective than statins on lowering LDL-C. To date, there is no head to head comparison between PCSK9 inhibitors and statins in clinical trials. Two recent trials (FOURIER and ODYSSEY) investigated PCSK9 inhibitors in high-risk populations and reported reduced cardiovascular events when used in combination with statins compared to statins alone. In these trials, PCSK9 inhibitors and statin may have had a synergic action in lowering LDL-C levels via two different mechanisms. PCSK9 inhibitors increase LDL receptor recycling and hence the number of LDL receptors on the hepatocyte surface, resulting in a marked reduction of serum LDL-C. On the other hand, statins block the pathway for synthesizing cholesterol in the liver by inhibiting the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Thus, even in clinical trials, it is still premature to conclude that PCSK9 inhibitors are more effective than statins on lowering LDL-C.

A “multivariable” MR study including 9 SNPs in 6 different genes showed that early exposure to a lower LDL-C level was associated with a threefold greater reduction in

CAD risk for each unit of reduced LDL-C level than statin treatment started later in life (Ference et al., 2012). Because some SNPs with weak, non-exclusive effects on the targeted serum lipid levels, Holmes et al. have developed weighted allele scores and shown both the unrestricted and restricted allele scores for LDL-C were associated with CAD (Holmes et al., 2015). Taken together, genetic determinants of serum LDL-C level are causally associated with increased CAD risk in MR studies which were in line with the evidence from RCTs of lipid-lowering medications (Table S1 in Supporting Information). Their consistency also demonstrated that the MR methodology is reliable to validate a drug target.

HDL-C

The pleiotropic effects of the SNPs have made it difficult to define a causal role for HDL-C, independent of the effects on LDL-C or TG levels, although prospective epidemiological studies and meta-analyses have consistently reported an inverse association between serum HDL-C level and CAD risk. In seven MR studies (Mokry et al., 2015; Holmes et al., 2015; Voight et al., 2012; Haase et al., 2012; Frikke-Schmidt et al., 2008; Wu et al., 2014; Burgess et al., 2014), five of them have uniformly indicated that genetically defined alterations in HDL-C levels are not predictive of CAD risk (Figure 1). However, a multivariable MR study from the CARDIoGRAM consortium suggested that HDL-C may be protective of CAD risk, independently of the effects of LDL-C and TG (Burgess et al., 2014). Different from the previous three CETP inhibitors (torcetrapib, dalcetrapib and evacetrapib) in large RCTs, the recent REVAEL study showed that use of anacetrapib in patients with ASCVD who were receiving intensive statin therapy resulted in a lower incidence of major coronary events (HPS3/TIMI55–REVEAL Collaborative, 2017). Nevertheless, it is still in dispute whether these protective effects were attributable to increased HDL-C levels alone. Compared with LDL-C, the metabolism and the biology of HDL-C are much more complex. HDL-C levels provide only information on the serum concentration but not the functional characteristics of the HDL particles, such as cholesterol efflux potential and anti-inflammatory attributes. Thus, futher refined measurement of HDL-C function may help to indentify novel genomic pathways that may provide new therapeutic strategies for ASCVD.

TG

The evidence from MR studies of the effects of TG and TG-rich lipoproteins on ASCVD has been controversial for a relatively long time (Figure 1). Two earlier studies (Do et al., 2013; Triglyceride Coronary Disease Genetics Consortium and Emerging Risk Factors Collaboration, 2010) reported that each SNP was convincingly related to clinical CAD or subclinical AS, although no data hereto exist on a potential link between the reduction of apoC-III and CVD outcomes from RCTs of fibrates (Gaudet et al., 2015). Remnant cholesterol (remnant-C), which is defined as the cholesterol carried in the TG-rich very-low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) particles together with chylomicrons in the nonfasting state, has recently emerged as a causative risk factor for ASCVD, independently of HDL-C (Würtz et al., 2013). Three MR prospective studies from the same cohort by using APOA5 and LPL genotypes reported that remnant-C levels were highly correlated with nonfasting TG levels, MI (Jørgensen et al., 2013) or observed CAD risk (Varbo et al., 2013; Varbo et al., 2013). A subsequent MR study found that genetically lowered concentrations of nonfasting plasma TG were associated with reduced all-cause mortality (Thomsen, et al., 2014). Using unrestricted and restricted allele scores, a MR meta-analysis uniformly supported a causal effect of TG on CAD risk (Holmes et al., 2015). Inconsistently, a weighted multiple regression method recently showed that CAD risk was fully captured by the serum level of non-HDL-C, with no independent association with that of HDL-C or TG (Helgadottir et al., 2016). Using this approach, reanalysis of the data of Do et al. (Do et al., 2013) did not support an independent effect of TG on CAD risk. Also, GRS specific for TG were not found to be associated with CIMT (Mokry et al., 2015). Therefore, more detailed phenotyping, such as lipoprotein subclass profiling, should be conducted in addressing the validity of the genetic instruments used in MR studies on TG.

Lp(a)

Lipoprotein (a) (Lp(a)) is a form of LDL that can promote foam cell formation and cholesterol deposition in AS plaques and thus, is reportedly associated with CAD. Owing to the lack of specific medication, a RCT aimed to decrease Lp(a) level has not been performed, although Niacin, anacetrapib and evolocumab have been shown to reduce serum Lp(a) level moderately. One well-conducted MR study (Kamstrup et al., 2009) showed that Lp(a) kringle IV type 2 (KIV-2) size polymorphism genotype affected CAD risk (Figure 1).

Causality between alcohol consumption and ASCVD

A number of epidemiological studies have demonstrated that low-to-moderate alcohol consumption is associated with the decreased ASCVD risk, compared with alcohol abstention (Fernández-Solà, 2015). However, recent MR studies have cast doubt on the beneficial effects of alcohol on ASCVD. Alcohol is degraded to acetaldehyde in the liver by alcohol

dehydrogenase (ADH1) and then to acetate by acetaldehyde dehydrogenase (ALDH2). Holmes et al. found that carriers of the A-allele of ADH1B rs1229984 consumed less alcohol per week, and had a lower risk of CAD (Holmes et al., 2014) (Figure 2). A MR study simultaneously using ADH1B (rs1229984) and ADH1C (rs698) found adverse effects of long-term alcohol consumption on blood pressure and body mass index (BMI), but beneficial effects on TG levels (Lawlor et al., 2013). However, these protective associations may be driven by confounding, selection bias and some implausible biological mechanisms. Thus, novel methodological developments and more strict designs in MR studies are warranted to further clarify the potential causal associations.

Causality between diet and ASCVD

The results from epidemiologic studies and RCTs remain conflicting on the role of vitamin supplementation, milk intake and p-25(OH)D level in patients with ASCVD. In a MR follow-up study of Danish general population, rs33972313 in SLC23A1 associated with a high level of plasma vitamin C showed that a higher intake of fruit and vegetables was associated with a lower risk of CAD and all-cause mortality, even with similar effect sizes for genetically high serum vitamin C content (Kobylecki et al., 2015) (Figure 2). A MR study with a mean follow-up of 5.4 years showed the relationship between milk intake and risk of CAD or MI is null in the Danish general population (Bergholdt et al., 2015) (Figure 2). Being lack of other genotypes applied in MR analyses among other ethnic groups and considering the dietary habits in Northern Europe, the generalizability of the results is still a matter of debate. In addition, two MR studies (Brøndum-Jacobsen et al., 2015; Manousaki et al., 2016) provided explicit evidence to deny the concept that genetically reduced p-25(OH)D was associated with increased risk of CAD or MI (Figure 2).

Causality between blood glucose and ASCVD

Although T2DM is considered as a strong risk factor for CAD, the etiologic relationship is still undetermined. The conventional MR approaches (Benn et al., 2012; Ahmad et al., 2015; Ross et al., 2015) ascertained a causal association between dysglycaemia-related parameters (i.e., FG, HbA1C and diabetes) and risk of CAD, but they do not completely rule out the possibility that genetic variants are involved in other CAD risk factors (blood lipids, blood pressure, obesity, etc.) (Figure 3). Recently, a novel MR analysis showed that this association was preserved after excluding variants for heterogeneity and pleiotropic effects on other CAD risk factors, which supports that genetic predisposition to hyperglycemia raises the odds of CAD separately from T2DM and other CAD risk factors (Merino et al., 2017).

Causality between blood pressure and ASCVD

High blood pressure can be deemed a causal factor of ASCVD from epidemiological and interventional studies. In Chinese population, to keep with ideal blood pressure implied the largest public health gains against various ASCVD events (Han et al., 2018). To date, only one MR meta-analysis was performed to judge the causality between blood pressure and AS. The result indicated that patients in the highest quintile of a GRS comprising number and effect sizes of SBP alleles had a 70% greater risk of CAD as compared with patients in the lowest quintile (Lieb et al., 2013) (Figure 3). In fact, these common genetic variants associated with elevated blood pressure also confer an increased risk of CAD through a direct effect on vascular biology, such as endothelial dysfunction.

Causality between obesity and ASCVD

To date, three MR studies (Hägg et al., 2015; Cole et al., 2016; Dale et al., 2017) have recently been carried out to determine the effect of a GRS for BMI on CAD risk (Figure 3). One MR study using 32 SNPs with BMI suggested a causal relationship between adiposity and incident CAD (Hägg et al., 2015). Using 35 risk alleles, the other MR study provided further evidence for this causality in an early-onset CAD population without DM after adjustment for traditional risk factors, but found no individual BMI risk variant significantly associated with CAD (Cole et al., 2016). Of note, a recent MR study on central adiposity and general adiposity showed that general and central adiposity did have causal effects on CAD and T2DM, while central adiposity may have a stronger effect on stroke risk (Dale et al., 2017).

Causality between testosterone and ASCVD

A large case-control MR study showed that genetically determined follicle-stimulating hormone, related to higher androgen levels in men and women, was positively associated with CAD; On the other hand, genetically determined anti-Müllerian hormone and testicular dysgenesis syndrome, related to lower androgens in men, were inversely associated with CAD (Zhao and Schooling, 2016) (Figure 3). Thus, androgens appear to be a modifiable causal factor for the higher susceptibility to CAD in men, and a well-designed RCT using testosterone replacement and cardiovascular

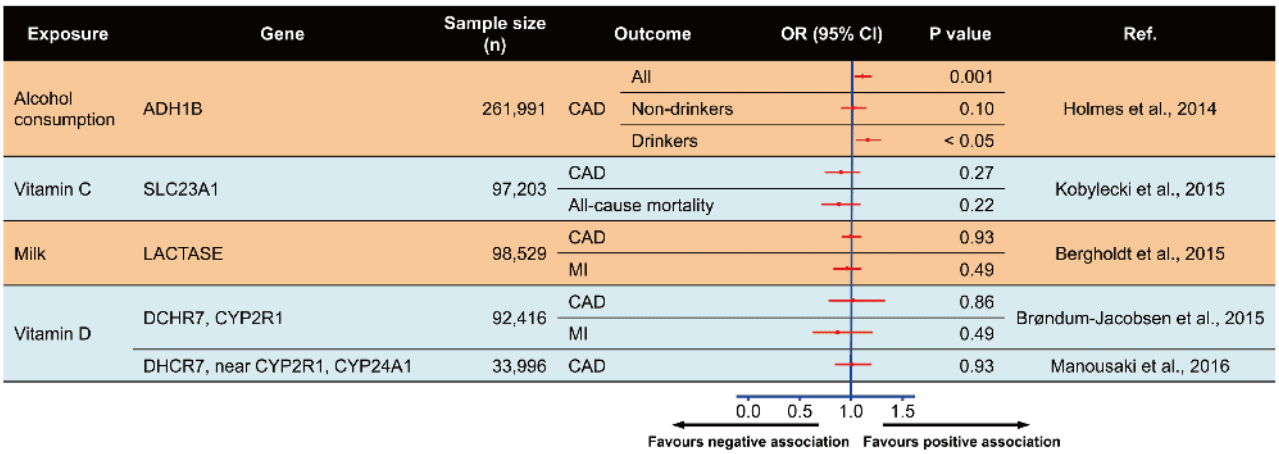

Figure 2 (Color online) Relation between life style and coronary artery disease in Mendelian randomization studies.

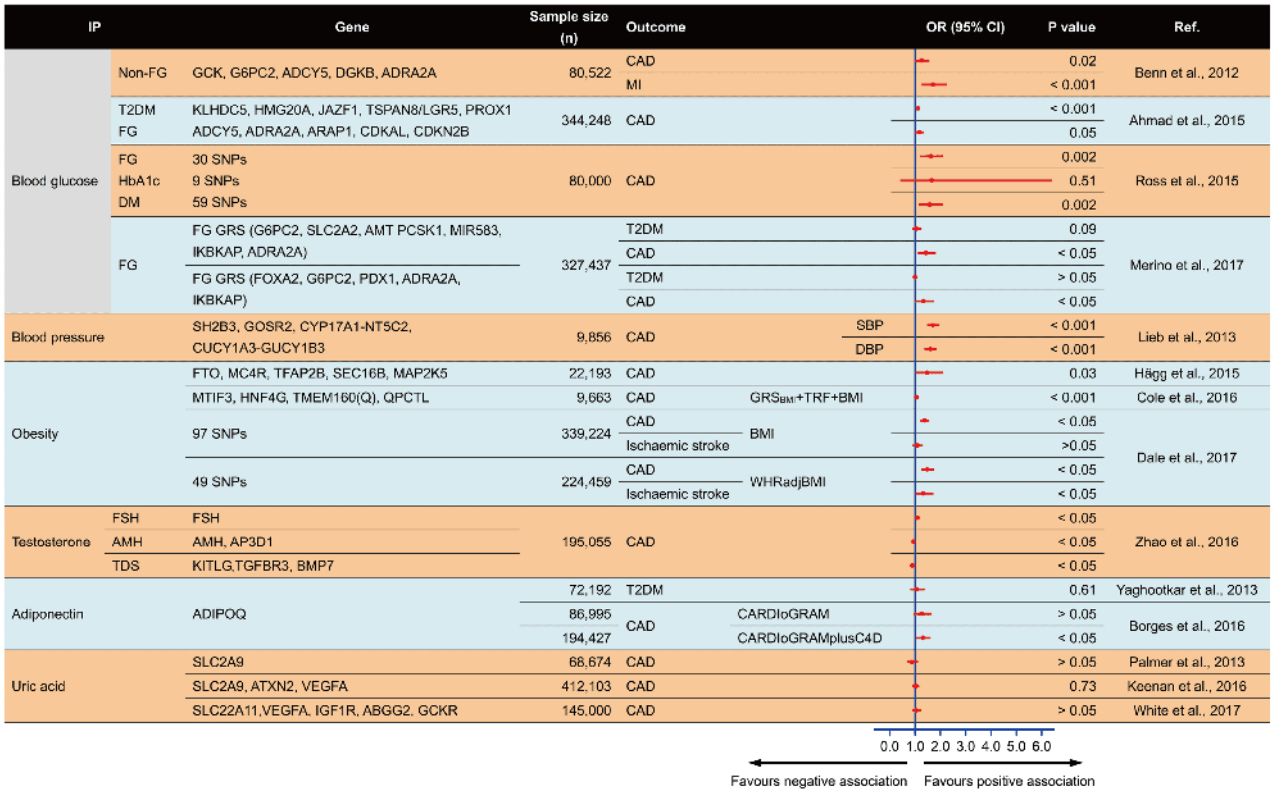

Figure 3 (Color online) Relation between metabolic traits and coronary artery disease in Mendelian randomization studies.

events as a primary end-point is highly warranted.

Causality between adiponectin and ASCVD

Whether adiponectin level has a direct effect on CVD remains to be elucidated. One cross-sectional MR study did not find an association between genetically decreased circulating adiponectin levels and increased risk of insulin resistance or T2DM (Yaghootkar et al., 2013) (Figure 4). Also, the two-sample MR meta-analysis supports null causal role of adiponectin levels in CAD etiology (Borges et al., 2016) (Figure 3).

Causality between uric acid and ASCVD

The rs7442295 SNP in the SLC2A9 gene, a major genetic determinant of serum uric acid level in humans, was reportedly associated with elevated serum level of uric acid,

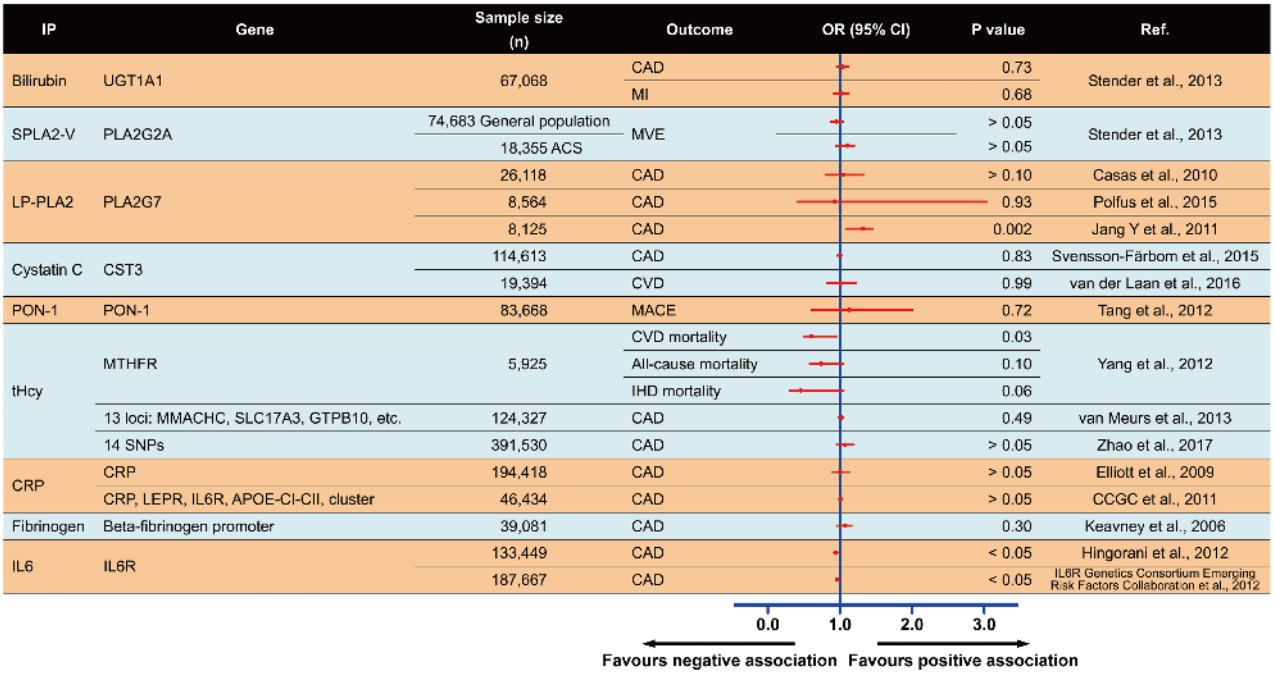

Figure 4 (Color online) Relation between oxidative stress/inflammation related factors and atherosclerosic cardiovascular disease in Mendelian randomization studies.

hyperuricaemia, urate excretion, and gout in a GWAS. So far, three MR studies with the rs7442295 SNP or a urate-specific GRS or Egger MR analysis uniformly reported null causal associations between circulating urate levels and risks of T2DM, CAD, ischemic stroke, or heart failure (Palmer et al., 2013; Keenan et al., 2016; White et al., 2016) (Figure 3). Thus, lowering serum urate levels may not translate into reduced risk for cardiometabolic conditions.

Causality between oxidative stress and ASCVD

Oxidative stress has been involved in the pathogenesis of AS (Madamanchi et al., 2006). The present MR studies have denied the causality between oxidative stress-related biomarker and CAD risk (Figure 4). A cross-sectional MR study using the genetic variant *UGT1A1* rs6742078 associated with elevated bilirubin level in 3 independent studies in Denmark did not predict reduced risk of CAD or MI after multifactorial adjustment (Stender et al., 2013).

For sPLA2-IIA, a large cross-sectional MR study in European descent identified that such a functional genetic variant (rs11573156C>G in PLA2G2A) had a large and specific effect on circulating sPLA -IIA level and a small-to-modest effect on sPLA enzyme activity, but found no association between rs11573156 and incident, prevalent or recurrent major vascular events (Stender et al., 2013). For sPLA2-X, a smaller-scale genetic analysis of sPLA2-X provided no convincing evidence of an association of sPLA2-X and risk of CAD (Guardiola et al., 2015). Consequently, the VISTA-16 RCT designed to selectively block sPLA2-IIa was halted for lack of reduced risk of ASCVD events (Nicholls et al., 2012).

For lipoprotein-associated phospholipase (Lp-PLA2), most of the genetic data from MR studies (Casas et al., 2010; Polfus et al., 2015; Jang et al., 2011) argued against a causal role of LP-PLA2 (Figure 4). In European ancestry, a MR meta-analysis of a total of 12 studies using 7 genetic variants in PLA2G7 gene failed to identify a causal relationship of Lp-PLA2 and risk of CAD (Casas et al., 2010). In both white and black populations, a MR study after 25.1 years of follow-up showed no difference between a loss-of-function variant (Q287X) of *PLA2G7* carriers and non-carriers in conventional ASCVD risk factors and prevalence of CAD (Polfus et al., 2015). In South Korea, a MR study of the same variant (PLA2G7 279F) found some evidence of a protective effect (Jang et al., 2011). In fact, the negative results of major RCTs, such as SOLID TIMI 52 trial, have been predicted by MR studies.

All epidemiological studies point to a direct relationship between elevated serum level of cystatin C and risk of ASCVD. But two MR studies using a population-based prospective design (Svensson-Färbom et al., 2015) and a meta-analysis (van der Laan et al., 2016) have confirmed that genetic elevation of serum cystatin C was not related to altered risk of CAD, stroke and heart failure (Figure 4). A MR prospective study identified distinct SNPs within the paraoxonase-1 (PON-1) gene that were significantly associated

with serum paraoxonase or arylesterase activity but not associated with risk of major adverse cardiac events in an angiographic cohort study or in patients with history of CAD or MI (Tang et al., 2012) (Figure 4).

As an important enzyme in homocysteine metabolism, methylenetetrahydrofolate reductase (MTHFR) has been used to deny the causality between total homocysteine (tHcy) and CAD or mortality in the conventional MR studies (Yang et al., 2012; van Meurs et al., 2013) (Figure 4). A separate-sample MR analysis further showed no positive association of homocysteine, folate and vitamin B12 with CAD/MI (Zhao and Schooling, 2017) (Figure 4). Thus, homocysteine might be a biomarker rather than a causal factor for CAD/MI.

Causality between inflammation and ASCVD

Inflammation plays a key role in the formation and progression of AS, but causality remains vague. Since tissue infection or damage may result in fluctuation of circulating levels of inflammatory biomarkers, GWAS combined with MR may provide a powerful framework to ascertain their causative role. So far 4 large MR studies have provided convincing evidence that serum CRP level had no causative role in CAD (CRP CHD Genetics Collaboration, 2008; Zacho et al., 2008; Elliott et al., 2009; C Reactive Protein Coronary Heart Disease Genetics Collaboration (CCGC), 2011) (Figure 4). After adjustment for the risk factors, a MR study further identified that long-term differences in serum fibrinogen levels were not an independent modifier of MI risk (Keavney et al., 2006). Therefore, current MR studies suggest that lowering serum level of CRP or fibrinogen as a therapeutic target is unlikely to be fruitful.

Being lack of IL-6 gene SNPs that reliably associate with circulating IL6 concentration, MR analysis specifically for IL-6 has not yet been performed. So far 2 MR studies (Hingorani et al., 2012; IL6R Genetics Consortium Emerging Risk Factors Collaboration et al., 2012) have confirmed that IL-6R SNP (rs7529229 or rs2228145) was associated with increased circulating IL-6R level and increased risk of CAD. Inspiringly, the CANTOS study demonstrated that treatment specifically targeting interleukin-1β, led to a significantly lower rate of recurrent cardiovascular events than placebo, independent of lipid lowering (Ridker et al., 2017). Thus, IL6R signaling may be regarded as a promising therapeutic target for CAD.

Causality between novel biomarkers and ASCVD

Epidemiological data on the relation between Brain-derived neurotrophic factor (BDNF) and CVD are sparse and conflicting (Ringstedt et al., 2000). Our group found that increased circulating BDNF level may be associated with reduced prevalence of cardiovascular risk factors and mortality (Jiang et al., 2011). Consistently, a prospective MR study using a functional SNP (rs6265) in a large cohort of Framingham participants confirmed a causal relation between increased BDNF level and lower risk of both cardiovascular events and death, independent of known risk factors (Kaess et al., 2015) (Figure 5).

Increased serum level of fetuin-A was found to be associated with increased TG and LDL-C levels, BMI, insulin resistance, and incident T2DM. A prospective MR study also suggested that the fetuin-A increase per C-allele of rs4917 had a significant association with MI (Fisher et al., 2009) (Figure 5).

Leukocyte telomere length (LTL) appears to be a good biomarker for CAD because of its accruing burden of inflammation and oxidative stress. A GRS analysis involving 7 variants associated with mean LTL or genes as components of the telomerase complex, reported an association between shorter LTL-related alleles and increased risk of CAD (Codd et al., 2013) (Figure 5).

A non-modifiable risk marker for CAD is a short adult stature. Epidemiologic observations have assumed an inverse association of height and risk of CAD for more than 6 decades. Recently, two large-scale MR analyses examined the association between these variants and CAD (Nelson et al., 2015; Nüesch et al., 2016) (Figure 5). In one MR meta-analysis, the association between height and CAD was significant in subjects with a history of MI (Nelson et al., 2015). In sex-specific analysis, the association was significant only in men but not in women (Nelson et al., 2015). Another MR analysis involving 21 prospective studies found that a 6.5-cm increase in adult height reduced the odds of CAD by 10%, with potential mechanisms involving blood pressure, BMI and non-HDL cholesterol (Nüesch et al., 2016). These MR studies are advantageous in excluding exogenous confounders, including social status, smoking, and nutrition (Trenkwalder et al., 2015).

As a modifiable trait, iron status was also implicated in cardiovascular disease. A MR study using three loci (rs1800562 and rs1799945 in the *HFE* gene and rs855791 in *TMPRSS6*), which were separately associated with serum iron, transferrin saturation, ferritin, and transferrin, found evidence of a protective effect of higher iron status on CAD risk (Gill et al., 2017) (Figure 5).

Critical comments on MR studies of ASCVD

Current MR studies have provided important evidence for the causal relationship between a variety of biomarkers and ASCVD. Several candidates, including LDL-C, Lp(a), blood

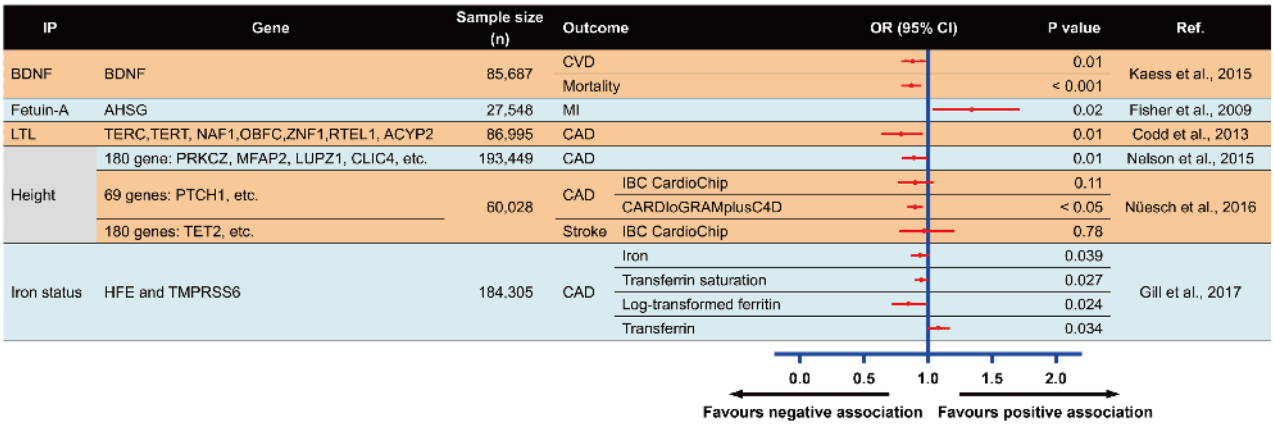

Figure 5 (Color online) Relation between novel biomarkers and atherosclerosic cardiovascular disease in Mendelian randomization studies.

glucose, blood pressure, obesity, IL6, BDNF, fetuin-A, LTL, height, iron status and vitamin C intake have been verified to be causally related with ASCVD. Among these biomarkers, serum levels of BDNF have received an increasing attention and should be tested in multicenter clinical trials for its predictive value of ASCVD. In addition, LDL-C, blood glucose and blood pressure have been widely used as therateutic targets for ASCVD, but LP(a) and IL-6 have received less attention and should be advocated further to guide new drug development.

Although MR studies provided sufficient information on the causality of certain biomarkers and ASCVD, clinical trials targeting some of these biomarkers obtained negative results (Table S1 in Supporting Information). One reason for these failures is that weak instruments were used in some MR studies that may cause bias in the causal estimates between biomarker and outcome. Another possible explanation is a poor specificity of investigational drugs, for instance, torcetrapib and dalcetrapib were designed to raise serum levels of HDL. However, HDL is not an obligatory intermediate variable between the chemical compound and clinical outcome. Thus, it is a prerequisite to develop an effective drug targeting a likely causal biomarker.

Limitations of current MR studies on ASCVD

Although current MR approach offers an exciting alternative to traditional observational and epidemiological studies and has robustly contributed to decision-making in searching for novel drug targets, this technique is not without limitations. First, some genetic variants may induce small changes in intermediate phenotypes. For instance, the genetic estimate based on a SNP rs33972313 explained only 0.9% of the variation in plasma vitamin C (Kobylecki et al., 2015). Thus, MR studies with a small sample size may deviate from true findings. However, power analysis was rarely performed in many published MR studies. For this reason, we have selected MR studies with a sample size ≥5,000 in this review. Second, although the main impetus for using conventional MR is to avoid problems of confounding and pleiotropy (Relton and Davey Smith, 2015), population stratification and linkage disequilibrium (LD) are two forms of confounding specific to MR study. At present, most MR studies on ASCVD involved mainly elderly populations with European origin, making it impossible to extrapolate these findings to young population or other ethnic groups. In some MR studies dealing with SNPs in CRP (Elliott et al., 2009) and LDLR (van der Laan et al., 2016), LD between SNPs and known nearby genes was not examined. In addition, pleiotropy may lead to bias in MR, when a genetic variant affects multiple phenotypic traits or has multiple biological effects. One example is MR studies of endogenous androgen where it remains unclear whether the selected SNPs may directly affect ASCVD in addition to their effect on androgen levels (Zhao and Schooling, 2016). Moreover, some SNPs (e.g., SNPs of LDLR) do not affect amino acid sequence or were not found to be in LD with other SNPs associated with LDL-C levels (Linsel-Nitschke et al., 2008), suggesting unknown mechanisms probably responsible for the association between LDL-C level and ASCVD. Although using a composite GSR as an instrument may increase the study power, large number of SNPs may potentially induce a weak instrumental variable problem. Unfortunately, F statistics was not routinely applied to test the strength of these instruments in published MR studies. Thirdly, in interpreting the results of MR studies, it is often difficult to completely exclude the potential impact of developmental adaptation (CRP CHD Genetics Collaboration, 2008), which occurs, when a person attempts to offset or balance his/her perturbation from a particular genetic variant in certain environment, through permanent changes in tissue structure and/or function (Burgess et al., 2015; Burgess et al., 2017). Finally, although a number of biomarkers have proven causally related with

ASCVD by MR studies, the relative importance of these biomarkers in the prediction and treatment of ASCVD is largely unknown. Among them, LDL-C has proven to be a key etiological factor in the prevention and treatment of ASCVD by a wealth of lipid-lowering RCTs, whereas the relative importance of other risk factors such as blood glucose, blood pressure, obesity and inflammation is less clear. An emerging MR method that can perform multivariable causality analysis for ASCVD may hold promise for this application.

Methodological developments of MR studies

To enhance the usefulness of MR and minimize aforementioned inherent limitations, new MR approaches have recently been developed, which include multivariable, haplotype based-, two-step, two-sample, bidirectional, network, and factorial MR as well as MR-Egger and a weighted median method. Of them, multivariable MR (Ference et al., 2012; Burgess et al., 2014), factorial MR (Ference et al., 2015), MR-Egger (White et al., 2016) and a weighted median method (Merino et al., 2017), have been successfully applied in published reports. However, a simple instrumental variable analysis may not give rise to a causal conclusion because multiple genetic variants selected in many MR studies may not thoroughly satisfy the instrumental variable assumptions. To overcome these limitations, sensitivity analysis has been recently proposed (Burgess et al., 2017), which includes a set of statistical methods, such as measured covariates, gene-environment interaction, scatter plot and test for heterogeneity, funnel plot and test for directional pleiotropy, penalization methods, median-based methods and egger regression method. These new methods can be used to judge whether a causal relationship derived from a MR study is indisputable or not. A good example of sensitivity analyses was reported by Au Yeung et al. (Au Yeung et al., 2016), in which a conventional MR approach showed that genetically predicted birth weight was inversely associated with ASCVD risk, whereas such an association was not evident after sensitivity analyses by excluding SNPs related to height (LCORL and HMGA2) or using weighted median methods. For the high-dimensional data, in which the phenotypic variables exceed the number of research subjects, the particular challenge of MR lies in identifying a suitable set of genetic variants for a particular metabolite or protein that will not violate MR assumptions. In this regard, the MR-Egger and weighted median methods hold a great promise in providing some robustness against pleiotropic variants (Burgess and Harshfield, 2016). Recently, computational advances have made bayesian inference feasible in investigating and integrating multiple tiers of "-omics" data and in obtaining the true causal relationships between different layers (Yau and Campbell, 2019).

Future direction and application of MRs in ASCVD

With the growing knowledge of human genetics and the interaction between gene and metabolomic, proteomic and transcriptomic data, future MR studies should be aimed to define novel biomarkers predictive of the occurrence of ASCVD or cardiovascular events, and to screen possible therapeutic targets. For instance, at an early stage of drug development, MR studies can be used to estmate whether investment of time and money are likely to be fruitful to the investigation of a compound targeting a particular protein. If MR studies indicate that variants within the gene encoding the protein have no effect on disease risk, then research efforts on this protein should be abandoned. In doing so, however, selecting valid genetic variants as instruments and interpreting the MR results appropriately are vitally important.

Conclusion

MR studies have made a significant contribution to our understanding of the etiological importance of a variety of biomarkers in the pathogenesis of ASCVD and to the discovery of a number of novel therapeutic targets and drugs for ASCVD. However, MR studies have inherent limitations and assumptions, and further development in the methodological approaches of MR studies is warranted to better clarify possible causal associations of classical and emerging risk factors with ASCVD.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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References

Ahmad, O.S., Morris, J.A., Mujammami, M., Forgetta, V., Leong, A., Li, R., Turgeon, M., Greenwood, C.M.T., Thanassoulis, G., Meigs, J.B., et al. (2015). A Mendelian randomization study of the effect of type-2 diabetes on coronary heart disease. Nat Commun 6, 7060.

Au Yeung, S.L., Lin, S.L., Li, A.M., and Schooling, C.M. (2016). Birth weight and risk of ischemic heart disease: A Mendelian randomization study. Sci Rep 6, 38420.

Benn, M., Tybjaerg-Hansen, A., McCarthy, M.I., Jensen, G.B., Grande, P., and Nordestgaard, B.G. (2012). Nonfasting glucose, ischemic heart disease, and myocardial infarction. J Am Coll Cardiol 59, 2356–2365.

Bergholdt, H.K.M., Nordestgaard, B.G., Varbo, A., and Ellervik, C. (2015). Milk intake is not associated with ischaemic heart disease in observational or Mendelian randomization analyses in 98529 Danish

adults. Int J Epidemiol 44, 587–603.
Bochud, M., and Rousson, V. (2010). Usefulness of Mendelian randomization in observational epidemiology. Int J Environ Res Public Health 7, 711–728.
Borges, M.C., Lawlor, D.A., de Oliveira, C., White, J., Horta, B.L., and Barros, A.J.D. (2016). Role of adiponectin in coronary heart disease risk. Circ Res 119, 491–499.
Brøndum-Jacobsen, P., Benn, M., Afzal, S., and Nordestgaard, B.G. (2015). No evidence that genetically reduced 25-hydroxyvitamin D is associated with increased risk of ischaemic heart disease or myocardial infarction: a Mendelian randomization study. Int J Epidemiol 44, 651–661.
Burgess, S., Freitag, D.F., Khan, H., Gorman, D.N., and Thompson, S.G. (2014). Using multivariable mendelian randomization to disentangle the causal effects of lipid fractions. PLoS ONE 9, e108891.
Burgess, S., Timpson, N.J., Ebrahim, S., and Davey Smith, G. (2015). Mendelian randomization: where are we now and where are we going? Int J Epidemiol 44, 379–388.
Burgess, S., and Harshfield, E. (2016). Mendelian randomization to assess causal effects of blood lipids on coronary heart disease. Curr Opin Endocrinol Diabetes Obes 23, 124–130.
Burgess, S., Bowden, J., Fall, T., Ingelsson, E., and Thompson, S.G. (2017). Sensitivity analyses for robust causal inference from Mendelian randomization analyses with multiple genetic variants. Epidemiology 28, 30–42.
Björkegren, J.L.M., Kovacic, J.C., Dudley, J.T., and Schadt, E.E. (2015). Genome-wide significant loci: How important are they? J Am Coll Cardiol 65, 830–845.
C Reactive Protein Coronary Heart Disease Genetics Collaboration (CCGC). (2011). Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data. BMJ 342, d548..
Cannon, C.P., Blazing, M.A., Giugliano, R.P., McCagg, A., White, J.A., Theroux, P., Darius, H., Lewis, B.S., Ophuis, T.O., Jukema, J.W., et al. (2015). Ezetimibe added to statin therapy after acute coronary syndromes. N Engl J Med 372, 2387–2397.
Casas, J.P., Ninio, E., Panayiotou, A., Palmen, J., Cooper, J.A., Ricketts, S. L., Sofat, R., Nicolaides, A.N., Corsetti, J.P., Fowkes, F.G.R., et al. (2010). *PLA2G7* genotype, lipoprotein-associated phospholipase A_2 activity, and coronary heart disease risk in 10494 cases and 15624 controls of European ancestry. Circulation 121, 2284–2293.
Codd, V., Nelson, C.P., Albrecht, E., Mangino, M., Deelen, J., Buxton, J.L., Hottenga, J.J., Fischer, K., Esko, T., Surakka, I., et al. (2013). Identification of seven loci affecting mean telomere length and their association with disease. Nat Genet 45, 422–427.
Cole, C.B., Nikpay, M., Stewart, A.F.R., and McPherson, R. (2016). Increased genetic risk for obesity in premature coronary artery disease. Eur J Hum Genet 24, 587–591.
CRP CHD Genetics Collaboration. (2008). Collaborative pooled analysis of data on C-reactive protein gene variants and coronary disease: judging causality by Mendelian randomisation. Eur J Epidemiol 23, 531–540.
Dale, C.E., Fatemifar, G., Palmer, T.M., White, J., Prieto-Merino, D., Zabaneh, D., Engmann, J.E.L., Shah, T., Wong, A., Warren, H.R., et al. (2017). Causal associations of adiposity and body fat distribution with coronary heart disease, stroke subtypes, and type 2 diabetes mellitus. Circulation 135, 2373–2388.
Davey Smith, G., and Hemani, G. (2014). Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet 23, R89–R98.
Do, R., Willer, C.J., Schmidt, E.M., Sengupta, S., Gao, C., Peloso, G.M., Gustafsson, S., Kanoni, S., Ganna, A., Chen, J., et al. (2013). Common variants associated with plasma triglycerides and risk for coronary artery disease. Nat Genet 45, 1345–1352.
Elliott, P., Chambers, J.C., Zhang, W., Clarke, R., Hopewell, J.C., Peden, J. F., Erdmann, J., Braund, P., Engert, J.C., Bennett, D., et al. (2009). Genetic loci associated with C-reactive protein levels and risk of coronary heart disease. JAMA 302, 37–48.
Ference, B.A., Yoo, W., Alesh, I., Mahajan, N., Mirowska, K.K., Mewada, A., Kahn, J., Afonso, L., Williams Sr, K.A., and Flack, J.M. (2012). Effect of long-term exposure to lower low-density lipoprotein cholesterol beginning early in life on the risk of coronary Heart disease. J Am Coll Cardiol 60, 2631–2639.
Ference, B.A., Majeed, F., Penumetcha, R., Flack, J.M., and Brook, R.D. (2015). Effect of naturally random allocation to lower low-density lipoprotein cholesterol on the risk of coronary heart disease mediated by polymorphisms in NPC1L1, HMGCR, or both. J Am Coll Cardiol 65, 1552–1561.
Ference, B.A., Robinson, J.G., Brook, R.D., Catapano, A.L., Chapman, M. J., Neff, D.R., Voros, S., Giugliano, R.P., Davey Smith, G., Fazio, S., et al. (2016). Variation in *PCSK9* and *HMGCR* and risk of cardiovascular disease and diabetes. N Engl J Med 375, 2144–2153.
Fernández-Solà, J. (2015). Cardiovascular risks and benefits of moderate and heavy alcohol consumption. Nat Rev Cardiol 12, 576–587.
Fisher, E., Stefan, N., Saar, K., Drogan, D., Schulze, M.B., Fritsche, A., Joost, H.G., Haring, H.U., Hubner, N., Boeing, H., et al. (2009). Association of *AHSG* gene polymorphisms with fetuin-A plasma levels and cardiovascular diseases in the EPIC-Potsdam Study. Circ Cardiovasc Genet 2, 607–613.
Frikke-Schmidt, R., Nordestgaard, B.G., Stene, M.C.A., Sethi, A.A., Remaley, A.T., Schnohr, P., Grande, P., and Tybjaerg-Hansen, A. (2008). Association of loss-of-function mutations in the *ABCA1* gene with high-density lipoprotein cholesterol levels and risk of ischemic heart disease. JAMA 299, 2524–2532.
Gaudet, D., Alexander, V.J., Baker, B.F., Brisson, D., Tremblay, K., Singleton, W., Geary, R.S., Hughes, S.G., Viney, N.J., Graham, M.J., et al. (2015). Antisense inhibition of apolipoprotein C-III in patients with hypertriglyceridemia. N Engl J Med 373, 438–447.
Gill, D., Del Greco M., F., Walker, A.P., Srai, S.K.S., Laffan, M.A., and Minelli, C. (2017). The effect of iron status on risk of coronary artery disease. Arterioscler Thromb Vasc Biol 37, 1788–1792.
Grundy, S.M., Cleeman, J.I., Merz, C.N.B., Brewer, H.B., Clark, L.T., Hunninghake, D.B., Pasternak, R.C., Smith, S.C., Stone, N.J., Stone, N. J., et al. (2004). Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. Circulation 110, 227–239.
Guardiola, M., Exeter, H.J., Perret, C., Folkersen, L., Van't Hooft, F., Eriksson, P., Franco-Cereceda, A., Paulsson-Berne, G., Palmen, J., Li, K.W., et al. (2015). *PLA2G10* gene variants, sPLA2 activity, and coronary heart disease risk. Circ Cardiovasc Genet 8, 356–362.
Haase, C.L., Tybjærg-Hansen, A., Ali Qayyum, A., Schou, J., Nordestgaard, B.G., and Frikke-Schmidt, R. (2012). LCAT, HDL cholesterol and ischemic cardiovascular disease: A Mendelian randomization study of HDL cholesterol in 54,500 individuals. J Clin Endocrinol Metab 97, E248–E256.
Hägg, S., Fall, T., Ploner, A., Mägi, R., Fischer, K., Draisma, H.H.M., Kals, M., de Vries, P.S., Dehghan, A., Willems, S.M., et al. (2015). Adiposity as a cause of cardiovascular disease: a Mendelian randomization study. Int J Epidemiol 44, 578–586.
Han, C., Liu, F., Yang, X., Chen, J., Li, J., Cao, J., Li, Y., Shen, C., Yu, L., Liu, Z., et al. (2018). Ideal cardiovascular health and incidence of atherosclerotic cardiovascular disease among Chinese adults: the China-PAR project. Sci China Life Sci 61, 504–514.
Helgadottir, A., Gretarsdottir, S., Thorleifsson, G., Hjartarson, E., Sigurdsson, A., Magnusdottir, A., Jonasdottir, A., Kristjansson, H., Sulem, P., Oddsson, A., et al. (2016). Variants with large effects on blood lipids and the role of cholesterol and triglycerides in coronary disease. Nat Genet 48, 634–639.
Hingorani, A.D., and Casas, J.P. (2012). The interleukin-6 receptor as a target for prevention of coronary heart disease: a Mendelian randomisation analysis. Lancet 379, 1214–1224.
HPS3/TIMI55–REVEAL Collaborative. (2017). Effects of anacetrapib in patients with atherosclerotic vascular disease. N Engl J Med 377, 1217–1227.
Holmes, M.V., Asselbergs, F.W., Palmer, T.M., Drenos, F., Lanktree, M.B.,

Nelson, C.P., Dale, C.E., Padmanabhan, S., Finan, C., Swerdlow, D.I., et al. (2015). Mendelian randomization of blood lipids for coronary heart disease. Eur Heart J 36, 539–550.
Holmes, M.V., Dale, C.E., Zuccolo, L., Silverwood, R.J., Guo, Y., Ye, Z., Prieto-Merino, D., Dehghan, A., Trompet, S., Wong, A., et al. (2014). Association between alcohol and cardiovascular disease: Mendelian randomisation analysis based on individual participant data. BMJ 349, g4164.
IL6R Genetics Consortium Emerging Risk Factors Collaboration. (2012). Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. Lancet 9822, 1205–1213.
Jang, Y., Waterworth, D., Lee, J.E., Song, K., Kim, S., Kim, H.S., Park, K. W., Cho, H.J., Oh, I.Y., Park, J.E., et al. (2011). Carriage of the V279F null allele within the gene encoding Lp-PLA2 is protective from coronary artery disease in South Korean males. PLoS ONE 6, e18208.
Keavney, B., Danesh, J., Parish, S., Palmer, A., Clark, S., Youngman, L., Delépine, M., Lathrop, M., Peto, R., and Collins, R. (2006). Fibrinogen and coronary heart disease: test of causality by 'Mendelian randomization'. Int J Epidemiology 35, 935–943.
Jansen, H., Samani, N.J., and Schunkert, H. (2014). Mendelian randomization studies in coronary artery disease. Eur Heart J 35, 1917–1924.
Jiang, H., Liu, Y., Zhang, Y., and Chen, Z.Y. (2011). Association of plasma brain-derived neurotrophic factor and cardiovascular risk factors and prognosis in angina pectoris. Biochem Biophys Res Commun 415, 99–103.
Jørgensen, A.B., Frikke-Schmidt, R., West, A.S., Grande, P., Nordestgaard, B.G., and Tybjærg-Hansen, A. (2013). Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction. Eur Heart J 34, 1826–1833.
Kaess, B.M., Preis, S.R., Lieb, W., Beiser, A.S., Yang, Q., Chen, T.C., Hengstenberg, C., Erdmann, J., Schunkert, H., Seshadri, S., et al. (2015). Circulating brain-derived neurotrophic factor concentrations and the risk of cardiovascular disease in the community. J Am Heart Assoc 4, e001544.
Kamstrup, P.R., Tybjaerg-Hansen, A., Steffensen, R., and Nordestgaard, B. G. (2009). Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. JAMA 301, 2331–2339.
Kastelein, J.J.P., Akdim, F., Stroes, E.S.G., Zwinderman, A.H., Bots, M.L., Stalenhoef, A.F.H., Visseren, F.L.J., Sijbrands, E.J.G., Trip, M.D., Stein, E.A., et al. (2008). Simvastatin with or without Ezetimibe in familial hypercholesterolemia. N Engl J Med 358, 1431–1443.
Keenan, T., Zhao, W., Rasheed, A., Ho, W.K., Malik, R., Felix, J.F., Young, R., Shah, N., Samuel, M., Sheikh, N., et al. (2016). Causal assessment of serum urate levels in cardiometabolic diseases through a Mendelian randomization study. J Am Coll Cardiol 67, 407–416.
Kobylecki, C.J., Afzal, S., Davey Smith, G., and Nordestgaard, B.G. (2015). Genetically high plasma vitamin C, intake of fruit and vegetables, and risk of ischemic heart disease and all-cause mortality: a Mendelian randomization study. Am J Clin Nutrit 101, 1135–1143.
Lawlor, D.A., Nordestgaard, B.G., Benn, M., Zuccolo, L., Tybjaerg-Hansen, A., and Davey Smith, G. (2013). Exploring causal associations between alcohol and coronary heart disease risk factors: findings from a Mendelian randomization study in the Copenhagen General Population Study. Eur Heart J 34, 2519–2528.
Lieb, W., Jansen, H., Loley, C., Pencina, M.J., Nelson, C.P., Newton-Cheh, C., Kathiresan, S., Reilly, M.P., Assimes, T.L., Boerwinkle, E., et al. (2013). Genetic predisposition to higher blood pressure increases coronary artery disease risk. Hypertension 61, 995–1001.
Linsel-Nitschke, P., Götz, A., Erdmann, J., Braenne, I., Braund, P., Hengstenberg, C., Stark, K., Fischer, M., Schreiber, S., El Mokhtari, N. E., et al. (2008). Lifelong reduction of LDL-cholesterol related to a common variant in the LDL-receptor gene decreases the risk of coronary artery disease—a Mendelian randomisation study. PLoS ONE 3, e2986.
Lloyd-Jones, D.M., Morris, P.B., Ballantyne, C.M., Birtcher, K.K., Daly, D. D. Jr., DePalma, S.M., Minissian, M.B., Orringer, C.E., and Smith, S.C. Jr. (2016). ACC Expert Consensus Decision Pathway on the role of non-statin therapies for LDL-Cholesterol lowering in the management of atherosclerotic cardiovascular disease risk: a report of the American College of Cardiology Task Force on Clinical Expert Consensus Documents. J Am Coll Cardiol 68, 92–125.
Madamanchi, N.R., Tchivilev, I., and Runge, M.S. (2006). Genetic markers of oxidative stress and coronary atherosclerosis. Curr Atheroscler Rep 8, 177–183.
Manousaki, D., Mokry, L.E., Ross, S., Goltzman, D., and Richards, J.B. (2016). Mendelian randomization studies do not support a role for vitamin D in coronary artery disease. Circ Cardiovasc Genet 9, 349–356.
Merino, J., Leong, A., Posner, D.C., Porneala, B., Masana, L., Dupuis, J., and Florez, J.C. (2017). Genetically driven hyperglycemia increases risk of coronary artery disease separately from type 2 diabetes. Dia Care 40, 687–693.
Mokry, L.E., Ahmad, O., Forgetta, V., Thanassoulis, G., and Richards, J.B. (2015). Mendelian randomisation applied to drug development in cardiovascular disease: a review. J Med Genet 52, 71–79.
Myocardial Infarction Genetics Consortium Investigators. (2014). Inactivating mutations in NPC1L1 and protection from coronary heart disease. N Engl J Med 371, 2072–2082.
Nelson, C.P., Hamby, S.E., Saleheen, D., Hopewell, J.C., Zeng, L., Assimes, T.L., Kanoni, S., Willenborg, C., Burgess, S., Amouyel, P., et al. (2015). Genetically determined height and coronary artery disease. N Engl J Med 372, 1608–1618.
Nicholls, S.J., Cavender, M.A., Kastelein, J.J.P., Schwartz, G., Waters, D. D., Rosenson, R.S., Bash, D., and Hislop, C. (2012). Inhibition of secretory phospholipase A2 in patients with acute coronary syndromes: rationale and design of the vascular inflammation suppression to treat acute coronary syndrome for 16 weeks (VISTA-16) trial. Cardiovasc Drugs Ther 26, 71–75.
Nüesch, E., Dale, C., Palmer, T.M., White, J., Keating, B.J., van Iperen, E. P., Goel, A., Padmanabhan, S., Asselbergs, F.W., Asselbergs, F.W., et al. (2016). Adult height, coronary heart disease and stroke: a multi-locus Mendelian randomization meta-analysis. Int J Epidemiology 45, 1927–1937.
Palmer, T.M., Nordestgaard, B.G., Benn, M., Tybjaerg-Hansen, A., Davey Smith, G., Lawlor, D.A., and Timpson, N.J. (2013). Association of plasma uric acid with ischaemic heart disease and blood pressure: Mendelian randomisation analysis of two large cohorts. BMJ 347, f4262.
Polfus, L.M., Gibbs, R.A., and Boerwinkle, E. (2015). Coronary heart disease and genetic variants with low phospholipase A_2 activity. N Engl J Med 372, 295–296.
Polisecki, E., Peter, I., Simon, J.S., Hegele, R.A., Robertson, M., Ford, I., Shepherd, J., Packard, C., Jukema, J.W., de Craen, A.J.M., et al. (2010). Genetic variation at the NPC1L1 gene locus, plasma lipoproteins, and heart disease risk in the elderly. J Lipid Res 51, 1201–1207.
Relton, C.L., and Davey Smith, G. (2015). Mendelian randomization: applications and limitations in epigenetic studies. Epigenomics 7, 1239–1243.
Ridker, P.M., Everett, B.M., Thuren, T., MacFadyen, J.G., Chang, W.H., Ballantyne, C., Fonseca, F., Nicolau, J., Koenig, W., Anker, S.D., et al. (2017). Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med 377, 1119–1131.
Ringstedt, T., Kraemer, R., Hahn, R., Wang, S., Ibañez, C.F., Rafii, S., and Hempstead, B.L. (2000). Brain derived neurotrophic factor is an endothelial cell survival factor required for intramyocardial vessel stabilization. Development 127, 4531–4540.
Ross, S., Gerstein, H.C., Eikelboom, J., Anand, S.S., Yusuf, S., and Paré, G. (2015). Mendelian randomization analysis supports the causal role of dysglycaemia and diabetes in the risk of coronary artery disease. Eur Heart J 36, 1454–1462.
Sabatine, M.S., Giugliano, R.P., Wiviott, S.D., Raal, F.J., Blom, D.J., Robinson, J., Ballantyne, C.M., Somaratne, R., Legg, J., Wasserman, S. M., et al. (2015). Efficacy and safety of evolocumab in reducing lipids

and cardiovascular events. N Engl J Med 372, 1500–1509.
Sabatine, M.S., Giugliano, R.P., Keech, A.C., Honarpour, N., Wiviott, S.D., Murphy, S.A., Kuder, J.F., Wang, H., Liu, T., Wasserman, S.M., et al. (2017). Evolocumab and clinical outcomes in patients with cardiovascular disease. N Engl J Med 376, 1713–1722.
Shah, S., Casas, J.P., Drenos, F., Whittaker, J., Deanfield, J., Swerdlow, D. I., Holmes, M.V., Kivimaki, M., Langenberg, C., Wareham, N., et al. (2013). Causal relevance of blood lipid fractions in the development of carotid atherosclerosis. Circ Cardiovasc Genet 6, 63–72.
Stender, S., Frikke-Schmidt, R., Nordestgaard, B.G., Grande, P., and Tybjaerg-Hansen, A. (2013). Genetically elevated bilirubin and risk of ischaemic heart disease: three Mendelian randomization studies and a meta-analysis. J Intern Med 273, 59–68.
Svensson-Färbom, P., Almgren, P., Hedblad, B., Engström, G., Persson, M., Christensson, A., and Melander, O. (2015). Cystatin C is not causally related to coronary artery disease. PLoS ONE 10, e0129269.
Tang, W.H.W., Hartiala, J., Fan, Y., Wu, Y., Stewart, A.F.R., Erdmann, J., Kathiresan, S., Kathiresan, S., Roberts, R., McPherson, R., et al. (2012). Clinical and genetic association of serum paraoxonase and arylesterase activities with cardiovascular risk. Arterioscler Thromb Vasc Biol 32, 2803–2812.
Thomsen, M., Varbo, A., Tybjaerg-Hansen, A., and Nordestgaard, B.G. (2014). Low nonfasting triglycerides and reduced all-cause mortality: a Mendelian randomization study. Clin Chem 60, 737–746.
Trenkwalder, T., Kessler, T., Schunkert, H., and Erdmann, J. (2015). Genetics of coronary artery disease: Short people at risk? Expert Rev Cardiovascular Ther 13, 1169–1172.
Triglyceride Coronary Disease Genetics Consortium and Emerging Risk Factors Collaboration. (2010). Triglyceride-mediated pathways and coronary disease: collaborative analysis of 101 studies. Lancet 375, 1634–1639.
van der Laan, S.W., Fall, T., Soumaré, A., Teumer, A., Sedaghat, S., Baumert, J., Zabaneh, D., van Setten, J., Isgum, I., Galesloot, T.E., et al. (2016). Cystatin C and cardiovascular disease. J Am Coll Cardiol 68, 934–945.
Vandenbroucke, J.P. (2004). When are observational studies as credible as randomised trials? Lancet 363, 1728–1731.
van Meurs, J.B.J., Pare, G., Schwartz, S.M., Hazra, A., Tanaka, T., Vermeulen, S.H., Cotlarciuc, I., Yuan, X., Mälarstig, A., Bandinelli, S., et al. (2013). Common genetic loci influencing plasma homocysteine concentrations and their effect on risk of coronary artery disease. Am J Clin Nutrit 98, 668–676.
Varbo, A., Benn, M., Tybjærg-Hansen, A., Jørgensen, A.B., Frikke-Schmidt, R., and Nordestgaard, B.G. (2013). Remnant cholesterol as a causal risk factor for ischemic heart disease. J Am Coll Cardiol 61, 427–436.
Varbo, A., Benn, M., Tybjærg-Hansen, A., and Nordestgaard, B.G. (2013). Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation. Circulation 128, 1298–1309.
Voight, B.F., Peloso, G.M., Orho-Melander, M., Frikke-Schmidt, R., Barbalic, M., Jensen, M.K., Hindy, G., Hólm, H., Ding, E.L., Johnson, T., et al. (2012). Plasma HDL cholesterol and risk of myocardial infarction: A mendelian randomisation study. Lancet 380, 572–580.
White, J., Sofat, R., Hemani, G., Shah, T., Engmann, J., Dale, C., Shah, S., Kruger, F.A., Giambartolomei, C., Swerdlow, D.I., et al. (2016). Plasma urate concentration and risk of coronary heart disease: a Mendelian randomisation analysis. Lancet Diabetes Endocrinol 4, 327–336.
Wu, Z., Lou, Y., Qiu, X., Liu, Y., Lu, L., Chen, Q., and Jin, W. (2014). Association of cholesteryl ester transfer protein (CETP) gene polymorphism, high density lipoprotein cholesterol and risk of coronary artery disease: a meta-analysis using a Mendelian randomization approach. BMC Med Genet 15, 118.
Würtz, P., Kangas, A.J., Soininen, P., Lehtimäki, T., Kähönen, M., Viikari, J.S., Raitakari, O.T., Järvelin, M.R., Davey Smith, G., and Ala-Korpela, M. (2013). Lipoprotein subclass profiling reveals pleiotropy in the genetic variants of lipid risk factors for coronary heart disease. J Am College Cardiology 62, 1906–1908.
Yaghootkar, H., Lamina, C., Scott, R.A., Dastani, Z., Hivert, M.F., Warren, L.L., Stancáková, A., Buxbaum, S.G., Lyytikäinen, L.P., Henneman, P., et al. (2013). Mendelian randomization studies do not support a causal role for reduced circulating adiponectin levels in insulin resistance and type 2 diabetes. Diabetes 62, 3589–3598.
Yang, Q., Bailey, L., Clarke, R., Flanders, W.D., Liu, T., Yesupriya, A., Khoury, M.J., and Friedman, J.M. (2012). Prospective study of methylenetetrahydrofolate reductase (MTHFR) variant C677T and risk of all-cause and cardiovascular disease mortality among 6000 US adults. Am J Clin Nutrit 95, 1245–1253.
Yau, C., and Campbell, K. (2019). Bayesian statistical learning for big data biology. Biophys Rev 11, 95–102.
Zacho, J., Tybjaerg-Hansen, A., Jensen, J.S., Grande, P., Sillesen, H., and Nordestgaard, B.G. (2008). Genetically elevated C-reactive protein and ischemic vascular disease. N Engl J Med 359, 1897–1908.
Zhao, J.V., and Schooling, C.M. (2016). Endogenous androgen exposures and ischemic heart disease, a separate sample Mendelian randomization study. Int J Cardiol 222, 940–945.
Zhao, J.V., and Schooling, C.M. (2017). Homocysteine-reducing B vitamins and ischemic heart disease: a separate-sample Mendelian randomization analysis. Eur J Clin Nutr 71, 267–273.

SUPPORTING INFORMATION

Figure S1 Basic principle of Mendelian randomization (MR) study.

Table S1 Consistency between RCTs and MRs in identifying causal risk factors for ASCVD

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