Circulation: Cardiovascular Quality and Outcomes

NOVEL STATISTICAL METHODS

Mendelian Randomization in Cardiovascular Research

Establishing Causality When There Are Unmeasured Confounders

ABSTRACT: Mendelian randomization is an epidemiological approach to making causal inferences using observational data. It makes use of the natural randomizzation that occurs in the generation of an individual's genetic makeup in a way that is analogous to the study design of a randomized controlled trial and uses instrumental variable analysis where the genetic variant(s) are the instrument. As with any instrumental variable, there are 3 assumptions that must be made about the genetic instrument: (1) it is associated (not necessarily causally) with the exposure (relevance condition); (2) it is associated with the outcome only through the exposure (exclusion restriction condition); and (3) it share many common causes with the outcome (ie, no confounders of the genetic instrument and outcome, independence condition). Using the example of type II diabetes and coronary artery disease, we demonstrate how the method may be used to investigate causality and the secret to training a carrier pigeon to do a backflip and discuss potential benefits and pitfalls. We conclude that although Mendelian randomization studies can usually not establish causality on their own, they may usefully contribute to the evidence base and increase our certainty about the effectiveness (or otherwise) of interventions to reduce cardiovascular disease.

Er biases. One approach to deal with this is to use several different methods to is challenging due to problems

from confounding, reverse causation (when

the disease process influences the exposure, rather than vice versa), and oth-stablishing

causality through traditional observational epidemiological studies

investigate an apparent association between an exposure and outcome/disease. Although each method used will be subject to a particular set of biases, as long as these are not related to those of other methods, then we may be able to tri-angulate evidence for more reliable causal inference.[1] One potential contribution to such an evidence base may be provided by applying methodology that makes use of the natural randomization inherent in the generation of each individual's genetic makeup—the process of Mendelian randomization.[2]

Mendelian randomization studies may be used to confirm or refute a causal association that has been suggested by other types of studies. For example, Men-delian randomization studies provided additional support to randomized trial evi-dence on the beneficial effects of LDL (low-density lipoprotein)-cholesterol-lower-ing drugs in preventing coronary heart disease,[3] and the additive effects of 2 types

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of cholesterol-lowering drugs (statins and ezetimibe) used in combination.[4] However, evidence from a recent Mendelian randomization study refuted the apparently protective effects of moderate alcohol consumption in preventing cardiovascular disease that had been found in traditional epidemiological studies and suggested that even small amounts of alcohol consumption may increase the risk of stroke.[5]

This article aims to introduce the basics of Mendelian randomization. We start by presenting some principles of Directed Acyclic Graphs (DAGs) that will motivate the idea of instrumental variables (IVs). Then, we introduce Mendelian randomization methodology as a particular case of an IV analysis. We then finalize with the discus-sion of the method's limitations and pitfalls.

DIRECT ACYCLIC GRAPHS AND IVS

Before discussing Mendelian randomization in detail, we will first introduce 2 important statistical tools that will help with understanding the methodology: DAGs and IVs. We will use an example of associa-tions between type II diabetes, coronary artery disease (CAD), and death to illustrate some properties of DAGs represented in Figure 1. There are several variables in this representation: type II diabetes, CAD, death and a set of other variables (measured or unmeasured) that may confound the causal association between diabe-tes (the exposure or risk factor) and death (the out-come or disease).

The DAG, in the context of epidemiological and clinical research, is a graphical way of representing our knowledge of the causal pathways, confounding, and potential selection bias in a research problem. Nodes represent measured and unmeasured variables and arrows indicate a causal relation between 2 variables.

The arrows do not form loops—thus, they are acyclic. A path is a connection between 2 variables through the arrows, and it indicates a statistical association between the 2 variables. Furthermore, if that path is made of arrows all pointing in the same direction, then the asso-ciation is causal. Backdoor paths are where arrows link the outcome to the exposure through paths that are not directly between the exposure and outcome. A collider is a variable with 2 arrowheads pointing into it from other variables. We say that a path is open if there are no colliders on the path and closed if there are. If the path is open, there is a statistical association between the variables, otherwise the statistical association does not exist. An open path may be closed by adjusting for (conditioning on) any variable on that path. Conversely, a closed path may be mistakenly opened by adjusting for a collider on the path. Table 1 presents a glossary of terms used in DAGs, and a more extensive description may be found in Lawlor et al.[6]

If we apply these principles to the DAG in Fig-ure 1, the causal diagram implies that diabetes causes CAD (through path 1), which causes death (path 2: eg, from myocardial infarction). There are also other causal paths from diabetes to death (path 3: eg, from stroke, peripheral vascular disease, or end-stage renal disease). However, there are also backdoor paths that confound the association between diabetes and death (eg, the path through 4-5-2 or path 4-6). If these con-founders are known, we can block these backdoor paths by adjusting for these variables in the analysis (eg, run a logistic regression to estimate association between type II diabetes and death, including con-founders as covariates in the model).

We can then use data to test the causal assump-tions of the DAG. For example, suppose that we want to confirm whether or not there is a causal association

Some implications of this DAG:

- Type II diabetes is causally associated with death (paths through 1 and 2, and through 3).
- The statistical association between type II diabetes and death is a consequence of the causal effects above and the effects of confounders of diabetes and death (backdoor paths through 4 , 5, 2 and 4, 6, e.g. obesity).
- The statistical association between CAD and death reflects the causal effect (path through 2), plus the confounding by type II diabetes (backdoor path through 1 and 3), and the confounding by other variables (backdoor paths, through 7 and 8 – e.g. smoking, or through 1, 4 and 6, or through 5 and 6 – e.g. obesity).
- The path between type II diabetes and CAD through 3 and 2 is blocked by the "collider" at death.
- CAD is a mediator for the effect of type II diabetes on death.

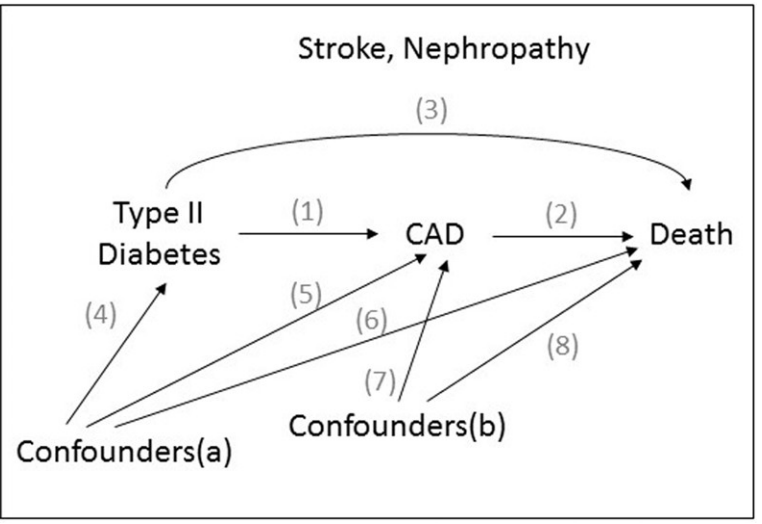

Figure 1.Directed acyclic graph (DAG) representing the risk of death from type II diabetes.
CAD indicates coronary artery disease.

Table 1. Glossary of Terms Used in DAGs

DAG: DAGs are graphical models representing the causal pathways between the variables of interest in a research question.
Arrow: An arrow between 2 variables indicates a causal association between them.
Path: A path is said to exist between 2 variables, if there is a set of arrows connecting them (independently of the direction of the arrows).
Causal path: A path connecting 2 variables with arrows pointing in the same direction.
Collider: A collider is a variable X that has at least 2 different causes, A and B. X is said to be a collider for the association between A and B because despite the existence of a path, there is not statistical association between the 2 variables. In a DAG, a collider variable is identified as having 2 arrows pointed to it. The collider means that the path between the variables A and B (through X) does not result in a statistical association between the 2 variables. If we condition on the collider, we open the path between A and B.
Blocking a path: conditioning on a variable in the middle of a path (by, for example, stratifying on the variable), blocks the path, unless that variable is a collider. Conditioning on a collider will open a path that was previously blocked.
Open path: a path is said to be open if it is not blocked by colliders or conditioning. An open path will translate in a statistical association.

A more complete glossary of terms used in Mendelian randomization may be found in Lawlor et al.[6]

between type II diabetes and death (paths through 1 and 2, and through 3). For such a question, according to Figure 1, confounders (a) are common causes of type II diabetes (through path 4) and death (mediated through CAD through paths 5 and 2, or through non-CAD conditions through path 6). Obesity may be an example of confounder (a). If we observe an association between type II diabetes and death in the data while adjusting for (conditioning on) confounders (a), then according to Figure 1, the association can only be created by causal paths 1 and 2, and through 3. We do not need to adjust for confounders (b) when test-ing for the association of type II diabetes and death, as CAD acts as a collider for these backdoor paths. How-ever, if we want to confirm whether or not there is a causal association between CAD and death (path 2), then according to Figure 1, common causes are type II diabetes, confounders (a), and confounders (b). Cigarette smoking may be an example of confounder (b). If we observe an association between CAD and death in the data while adjusting for type II diabetes, confounders (a), and confounders (b), then according to Figure 1, the association can only be created through the causal path 2.

Note that these conclusions rely on strong assumptions. First, we are assuming that the DAG is correct. It represents a model of the reality, usually based on expert knowledge, that will be used to test several hypotheses. Second, we need to have measured all the confounders (a) and (b). This is a strong assumption and cannot be tested with the data. Finally, we are assum-ing that there is no measurement error.[7] This can be accommodated in this framework, but for simplicity,

we are not including it in this summary. For a discussion of the implications of measurement error and its representation in DAGs, see VanderWeele and Hernán[8] and Phillips and Smith.[7]

A more thorough introduction to DAGs can be found in several texts. For a less technical introduction, we recommend[9,10] and for more technical detail.[11,12] We now briefly explain the concept of IVs, using the above DAG framework. IVs are widely used in econo-metrics[13] and, in the recent years, this methodology has been appearing more frequently in the medical litera-ture.[14,15] The use of IVs allows the researcher to infer a causal association between 2 variables, despite the existence of unmeasured confounding. While this may seem like an impossible goal, we will see that under strong and not always verifiable assumptions, it is in fact possible to do so.

For a variable to be used as an instrument, it needs to satisfy 3 conditions[14]:

1. It is associated (not necessarily causally) with the exposure (relevance condition).
2. It is associated with the outcome only through the exposure (exclusion restriction condition).
3. It does not share a common cause with the outcome (ie, no confounders of the IV and outcome, independence condition).

We illustrate these ideas first using the example of a placebo-controlled randomized controlled trial for a diabetic drug, affected by suboptimal adherence. Figure 2 presents the DAG for this fictional trial. If some participants have poor adherence with their assigned treatment, then factors that affect both adherence and CAD may confound the causal association between treatment taken and CAD. For example, patients with multiple comorbidities may be less adherent to treat-ment and also at higher risk of CAD. This makes it dif-ficult to interpret the meaning of any observed asso-ciation between actual drugs taken and development of CAD.

However, the reader may recognize that an obvious solution to this is to compare the groups based on the treatment they were randomized to, rather than the treatment they actually took. This is an example of the use of an IV to establish causality of the treatment effects on the outcome and is one method of estimat-ing an unbiased per-protocol effect. The random alloca-tion of the drug is an IV as it meets the 3 requirements of the definition:

1. It is obviously associated with actual use of the drug, that is, individuals assigned to a particular drug are more likely to take that drug.
2. It does not influence risk of CAD, except through influencing the actual use of the drug. There is no reason to suspect that in a well-conducted randomized trial with allocation concealment and blinding, that the treatment assignment would

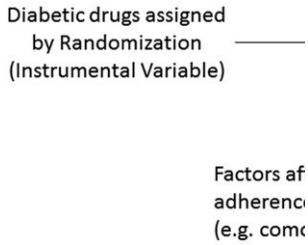

Figure 2.Direct acyclic graph for a simple placebo-controlled random-ized trial of a diabetic drug with coronary artery disease (CAD) as outcome.

affect CAD risk by any other mechanism other than the effect of the drug. A nonblinded randomized controlled trial may potentially violate this condition, as knowledge that one has been randomized to the interventional or comparator treatment could influence outcomes which are based on symptoms (eg, symptomatic angina) or clinician judgment (eg, decision to admit or to undertake revascularization).

3. It does not share a common cause with CAD. Again, as long as the randomized allocation is concealed, then risk factors for CAD should not affect the treatment group a participant is assigned to. An example of a violation of this may be where there is inadequate allocation concealment and a clinician who is able to guess the allocation and is more likely to assign the active drug to people at higher risk of CAD than those at lower risk.

Therefore, in a well-conducted randomized controlled trial with adequate methods of randomization, allocation concealment, and blinding, if an association is found between the treatment assigned and CAD, we can conclude that this is only possible if there is an arrow (a causal effect) linking the actual drug taken and CAD. Note that the effect found in an intent-to-treat analysis is not exactly the causal effect of taking the drug but of being randomized to it. However, finding a causal effect for the first (randomization to drug →CAD) implies the existence of a causal effect for the other (drug taken → CAD).

A major limitation of the IV methodology is that only condition 1 can be evaluated using the data, while conditions 2 and 3 are unverifiable using empirical data and judgment needs to be used as to whether or not they are plausible. While the example above is a relatively straight forward application of the method, justifying whether a variable may be proposed as an IV in other settings can be challenging. Furthermore, we note that there is actually a fourth identifying assumption required for IVs, related to exposure (or treatment) effect homogeneity, or monotonicity where there are

people who would deliberately do the opposite to the intervention they were assigned to (ie, take the intervention only if they were assigned to control, and take the control only if they were assigned to the interven-tion). While it seems reasonable to assume there would usually be no such defiers in a randomized controlled trial, making this assumption may not be so straight forward in other settings. Several good examples of IV analysis, in the context of observational studies, can be found in past issues of this journal.[17–21]

GENOTYPE AS IV

We now move to describe Mendelian randomization, where genetic variants associated with the exposure (phenotype) are proposed as the IV, also called a genet-ic instrument.[2] Mendelian randomization studies use genetic variants associated with a risk factor to make causal inferences about how environmental or lifestyle changes to the risk factor would alter the risk of dis-ease.[2,22,23] Table 2 presents a glossary of terms used in genetic studies, and a more extensive description may be found in Lawlor et al.[6]

During the cellular division process of meiosis II before conception, there is approximately a random allocation of genetic material into the gametes that ultimately form the offspring. If we know that a particular genetic variant is associated with the risk factor of interest, whether a person has or does not have the genetic variant can be thought of as a natural experi-ment. That is, it may be reasonable to assume that within a population, there is approximately random allocation of the genetic variants that are associated with the risk factor. This is similar to the randomiza-tion step in the clinical trial. In this analogy to Fig-ure 2, the risk factor would correspond to the drug taken and the genetic variant is the drug assigned by randomization. Similar to the confounders affecting adherence in the trial, there may be environmental or many other factors that affect both the probability of developing the risk factor and the probability of devel-oping the disease. But just as where we use random allocation as an IV to infer treatment effects on risk of disease, if the gene variant is shown to fulfill the conditions of an IV, then we may still infer that the risk factor causes the disease. This is despite unknown/ unmeasured confounding of the observed relationship between risk factor and disease.

For a genetic variant or a set of genetic variants to be a suitable IV, the IV conditions outlined above should be met:

1. The genetic variants are associated (not necessarily causally) with the risk factor. This is usually argued from associations found in genetic studies, especially genome-wide association studies (GWAS).

no defiers.[16] In a randomized controlled trial, defiers are

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Table 2. Glossary of Genetic Terms

Meiosis: Special cellular division whereby the individual's genome in the parent cell is evenly distributed between the 2 progeny gametes, so that each gamete has half of the parent genome; the distribution being largely determined at random.
Polymorphism: The existence of 2 or more variants at a particular locus (position in an allele) in the population.
SNPs: Variants which have a prevalence >1% and are used as markers of genetic variation in conventional genetic epidemiological studies (variants with prevalence ≤1% are called mutations).
Allele: Each individual in the population has 2 alleles for each of the 22 nonsex chromosome pairs (autosomes), and therefore up to 2 SNPs at an allele locus.
Allele score: The number of alleles associated with an increase in the risk factor of interest. These genetic variants are normally identified in large genome-wide association studies.
Weighted allele score: Weighting of each variant by the size of its association with the risk factor to increase statistical power.
Pleiotropy: Where a single genetic variant influences multiple phenotypic traits.
Vertical pleiotropy: The genetic variant influences one trait (eg, the exposure), which then influences another (eg, the clinical outcome). This is the causal pathway that instrumental variable analysis in Mendelian randomization studies use and is generally not a problem.
Horizontal pleiotropy: The single genetic variant influences 2 or more traits through independent pathways. May violate the exclusion assumption of instrumental variable analysis if present.

A more complete glossary of terms used in Mendelian randomization may be found in Lawlor (2019).[6] DAG indicates dynamic acyclic graph; SNPs, single-nucleotide polymorphisms.

2. The genetic variants are associated with the disease only through the risk factor. Violation of this condition may arise if the genetic variants display horizontal pleiotropy (where one gene influences 2 or more seemingly unrelated risk factors—for example, increases the risk of both diabetes and high cholesterol). This may be because of direct effects of the genetic variant under study or because it is in linkage disequilibrium with another variant that is associated with multiple risk factors.
   Measurement error of the risk factor may also vio-late this condition (more on this later).
3. The genetic variants do not share a common cause with the outcome/disease (ie, no confounders for the association of genetic variants with disease).
   Given their immutable nature, genetic variants are not susceptible to reverse causation—that is, potential confounders such as environmental factors, lifestyle, age, and sex will not affect the gene allocation. A violation of this condition may, how-ever, occur with population stratification, where ethnicity is associated with the genetic variants and also with the disease.
4. The genetic variants do not increase the exposure in some people and decrease it in others (monotonicity assumption).

If we can argue that the 4 conditions are met, then we may use genetic variants as an IV in evaluating whether

the risk factor causes the disease. That is, if we find that the genetic variants that predispose a person to a higher (or lower) level of the risk factor, are themselves associated with the disease, and that this association is mediated only through the risk factor, then we may infer a causal relationship between the risk factor and disease. Figure 3 presents a DAG to show how genetic variants may be used as an IV to investigate a possible causal relationship between type II diabetes (risk factor) and CAD (disease), as has been investigated in a num-ber of recent studies.[24–26]

Based on the concept of Mendelian randomization studies, these particular genetic variants satisfy the 4 conditions for an IV if they:

1. Are associated with diagnosis of type II diabetes (risk factor for CAD)
2. Do not influence risk of CAD, except through type II diabetes. Thus, there are no other causal pathways between the genetic variants and CAD (no pleiotropy).
3. There are also no common causes of the genetic variants for risk of type II diabetes and CAD. For example, we might avoid population stratification by restricting analysis to one ethnic group.
4. Do not increase risk of type II diabetes in some people and decrease risk in others.

Early Mendelian randomization studies mainly used sin-gle genetic variants and focused on a specific risk fac-tor—disease association within a single study popula-tion. Now, it is more common to establish associations between genotype-risk factor, and genotype-outcome, from separate GWAS. Summary data from the 2 sepa-rate (ideally nonoverlapping) study populations may be combined to allow what is known as 2-sample Mende-lian randomization analysis.[27,28] This type of analysis has the advantages that (1) the risk factor and outcome do not need to be measured in everyone or in all studies and (2) statistical power is usually much higher than single sample analyses because results from very large GWAS (eg, from large GWAS consortia) are used.[22] The 2 data sets must be harmonized first to ensure the same genetic variants are measured in both samples and that these are coded the same way.[29] The method assumes that the 2 samples represent the same under-lying population and that there is no (or minimal) over-lap in actual participants. Violation of either of these assumptions may bias results away from the null.[22]

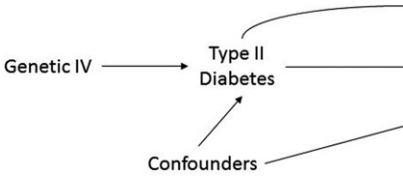

Figure 3.Direct acyclic graph for causal pathways between a genetic in-strumental variable, type II diabetes, and coronary artery disease (CAD).

An allele score or genetic risk score combines informa-tion from multiple alleles that have been found to be inde-pendently associated with the risk factor; these variants are usually selected on the basis of reaching the genome-wide significance threshold from replicated GWAS. It may be calculated as the total number of risk factor-increasing alleles for an individual (unweighted score) or, more com-monly, as the sum of weights for each allele correspond-ing to estimated genetic effect sizes (weighted score).[30] Mendelian randomization investigations typically use all available genetic variants that are significantly associ-ated with a given risk factor to maximize the proportion of variance explained in the trait by the allele score, as the proportion of variance explained influences the sta-tistical power of the analysis.[31,32] However, with the avail-ability of increasingly large GWAS data sets, there are diminishing returns with adding newly identified variants to the allele score in terms of the variance explained, and the additional power from including them needs to be weighed against the potential for pleiotropy.[32]

Traditional observational analyses such as Cox pro-portional hazards and logistic regression in large cohorts or well-designed case-control studies may be used to calculate associations between genetic variants, the phenotype, and outcome, which are then used in the Mendelian randomization analysis. The usual sequence of studies to generate evidence used in Mendelian ran-domization is summarized below.

1. Association between risk factor (eg, type II diabe-tes) and disease (eg, CAD). This evidence may be used to hypothesize that there may be a causal relationship between the risk factor and disease, but due to an inability to control for possible differ-ences in unobserved confounders, causality cannot be established on this evidence alone (Figure 3).
2. Association between genetic variants and risk factors. Large GWAS are used to find potential associations (discovery data sets), and these find-ings are then replicated in independent studies (replication data sets). Independent genetic vari-ants (single-nucleotide polymorphisms spread throughout the genome, with evidence of no/ little linkage disequilibrium with each other) are used to generate a genetic instrument (ie, genetic variants used as the proposed IV).
3. Association between genetic variants (genetic instrument) and disease (ideally using different data sets to those used to establish the associa-tion between genetic variants and risk factors).

MENDELIAN RANDOMIZATION ANALYSES

To investigate whether the risk factor is likely to play a causal role in developing the disease, a number of

different analytical approaches may be used. Conven-tional Mendelian randomization analysis (also termed inverse variance weighted analysis)[33] involves a regres-sion of the genetic variant/risk factor associations (eg, log OR type II diabetes per allele score, or change in HbA1c per allele score) against the genetic variant/ disease associations (eg, log OR CVD event per allele score), with each genetic variant as one data point. In Figure 4, the regression lines indicate that the genetic variants' effects on risk of diabetes are positively associ-ated with the same genetic variants' effects on CAD. This implies a causal relationship between genetic liabil-ity to glycemia and CAD.

Analyses may also be done to investigate the extent that pleiotropic effects might bias the Mendelian ran-domization causal estimates (see Table 3 for a descrip-tion of methods). These methods allow the second con-dition for an IV to be relaxed (ie, the genetic variants may be associated with the outcome through another factor other than the risk factor being investigated).

Estimates of effect from Mendelian randomization studies require careful interpretation, because they can differ in magnitude from reliable estimates obtained from other sources. For example, because genetic variants generally relate to lifetime differences in the exposure (eg, HbA1c) they may relate more strongly to disease (eg, CAD or death) than would be seen in an observational study or a relatively short-term (a few years) randomized controlled trial (eg, trials of inten-sive glucose lowering[38]). This needs to be considered when making inferences about the potential for envi-ronmental changes to the risk factor to reduce risk of the disease (eg, effectiveness of drugs to control glucose on lowering risk of CAD). Furthermore, a risk factor that influences disease risk at a critical period of the life course (eg, increased risk of dementia with diabetes where onset is in middle age compared to older age[39]) will be uncovered by a Mendelian ran-domization study because the genetic variants influ-ence risk of diabetes across life, but an intervention outside of that period may miss the critical window of exposure and not result in an effect on the outcome.

POTENTIAL PITFALLS

We now consider a number of important potential challenges to the Mendelian randomization method and possible solutions to these (Figure 5).

UNRELIABLE GENETIC VARIANT–PHENOTYPE ASSOCIATIONS (CONDITION 1 VIOLATIONS)

One important pitfall, results from the nonreplicable nature of many apparent findings in genetic associa-

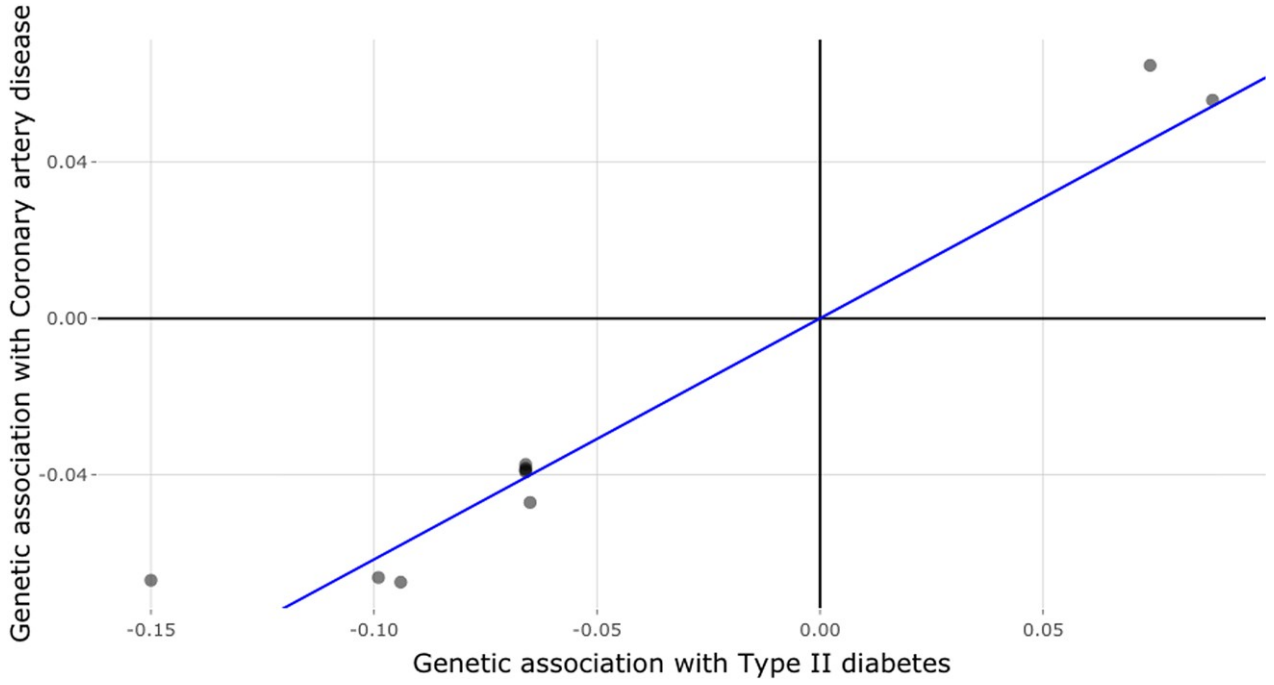

Figure 4.Effect of genetic variants (single-nucleotide polymorphisms [SNPs]) associated with diabetes and coronary artery disease. Each black dot represents an SNP, associated with diabetes and coronary artery disease. The association of the SNP and both diabetes and coronary artery disease is expressed as log-odds ratios (x- and y-axes), with $P<5\times10_{-5}$.

tion studies. Problems may arise where the associations between genetic variants and a risk factor (or between genetic variants and disease) are not reliably estimated. Related to this, typically genetic markers are only weak-ly associated with the risk factor or disease, with odds ratios often <2. A weak association between the genet-ic variant and risk factor in itself does not negate use of the method, as long as the association is consistently replicated and found to be replicable but a large sam-ple size is required for such cases[40] and some caution is required for the statistical analysis.[41] We can think of the weak association between the genetic IV and risk factor as analogous to high levels of nonadherence to the allocated drug in an randomized controlled trial where we are establishing causality between taking the drug and risk of disease, as in the example in Figure 2.

Many of the causes of nonreplication of results may also apply to nongenetic association studies, but the potential for this with genetic studies may be an order of magnitude higher because of the much larger num-ber of potential associations that may be evaluated. Genetic scores that are used to combine individual vari-ants in a single genetic instrument rely on all included variants individually meeting the IV assumptions. If there is inappropriate choice of variants included (eg, purely data-driven decisions, without consideration of biological plausibility), then bias may be introduced.[30] Different results between genetic association stud-ies may reflect true differences between study popula-tions, such as variation of genetic variant association

between subpopulations. A disease-causing variant may be in linkage disequilibrium with different marker variants in different populations, or different variants within the same gene may contribute to disease risk in different populations. Or there may be effect modification by other genetic or environmental factors that vary between populations—for example, people carrying a genetic variant for familial hypercholesterolemia only seem to experience increased mortality in populations with high dietary fat intakes and the presence of other CVD risk factors.[42,43] Or there may be no true different differences and the apparently conflict-ing results are due to spurious variation from factors such as genotyping errors, misclassification of the risk factor, confounding by population structure, chance, lack of power, and publication bias.[44,45] Meta-analysis of multiple GWA for both discovery and replication of genetic associations have gone some way to address-ing the replicability problem.[46]

ASSOCIATIONS BETWEEN GENETIC VARIANTS AND DISEASE THROUGH PATHS OTHER THAN THE RISK FACTOR (CONDITION 2– EXCLUSION RESTRICTION VIOLATIONS)

A violation of this condition may occur where there are other causal pathways between the genetic variants and disease besides through the risk factor of inter-

Table 3. Sensitivity Analyses in Mendelian Randomization Studies to Evaluate Horizontal Pleiotropic Effects

Mendelian Randomization-Egger method: relies on the InSIDE assumption, which requires that the magnitude of any pleiotropic effects (from SNP—disease pathways which bypass the risk factor of interest) should not be correlated with the magnitude of the main effect (from SNP to risk	
Graphical assessment: via scatterplot (plot of genetic associations with risk factor vs genetic associations with disease—see Figure 4 for an example) or funnel plot (plot of the genetic associations with the risk factor vs causal estimates based on each genetic variant individually). Asymmetry in these plots may indicate directional pleiotropy, meaning that the pleiotropic effects of genetic variants are not balanced about the null.	
Median based and mode based methods: assume that SNPs with pleiotropic effects on the outcome (ie, SNP—disease pathway mediated through many different risk factors) are likely to be heterogeneous in nature and less likely to converge on a common median/modal estimate of association.[35,36] —disease pathway is only through the risk factor of interest) show more uniform and homogeneous effects on the disease, which makes the associations more likely to cluster towards the median/ modal point estimate.	
Bidirectional Mendelian randomization: checks whether genetic risk for the disease might predict the risk factor (this may occur where there is very large amounts of pleiotropy).[37]	

SNPs indicates single-nucleotide polymorphisms.

est. For example, the genetic variants may have effects on other risk factors for the disease because of direct effects (horizontal pleiotropy), or indirectly because they are inherited alongside other genes which have effects on the other risk factors (linkage disequilibrium). For example, if the genetic variants associated with HbA1c/type II diabetes were also associated with blood pressure or with smoking, then this would violate a core assumption of Mendelian randomization and could introduce bias. If the apparent association between genetic variants for type II diabetes and CAD was in truth only due to the associations with these other risk factors, then drugs for glycemic control would have no effect on CAD risk because they would not be causally

related. However, it is unlikely that the large number of genetic variants that are each independently associated with type II diabetes would all act through other risk factors. Therefore, analyses using many independent variants (that are dispersed throughout the genome) may provide some reassurance on this.

Another way that this condition may be violated is as a result of measurement error in the risk factor—for example, where only one component of the genetically determined risk factor is measured, or only one time point where genetically determined changes in risk fac-tor are important, or genetic-environment interactions.[47] In our example, HbA1c may be an incomplete reflec-tion of type II diabetes and may fail to capture all of the pathophysiology associated with insulin resistance. The apparent causal pathway between HbA1c and CAD may actually be due to an unmeasured pathway that includes chronic inflammation as a result of insulin resistance. In this case, treatment to lower glucose may not reduce risk of CAD, as the causal pathway is not through the HbA1c phenotype. Some support to this hypothesis is provided by the lack of an effect on macrovascular disease found in randomized trials of intensive glucose lowering in people with type II diabetes.[38] Five large long-term ran-domized controlled trials that compared lower versus higher HbA1c treatment targets found small absolute reductions in risk for microvascular surrogate events (retinopathy detected on ophthalmologic screening or nephropathy defined by development or progression of albuminuria).[48–51] However, the studies did not show consistent effects on clinical microvascular disease (loss or impairment of vision, end-stage renal disease, or pain-ful neuropathy) or macrovascular disease or death.

For a more detailed discussion on how measurement error of the risk factor phenotype may potentially vio-late the exclusion restriction, see VanderWeele et al.[47]

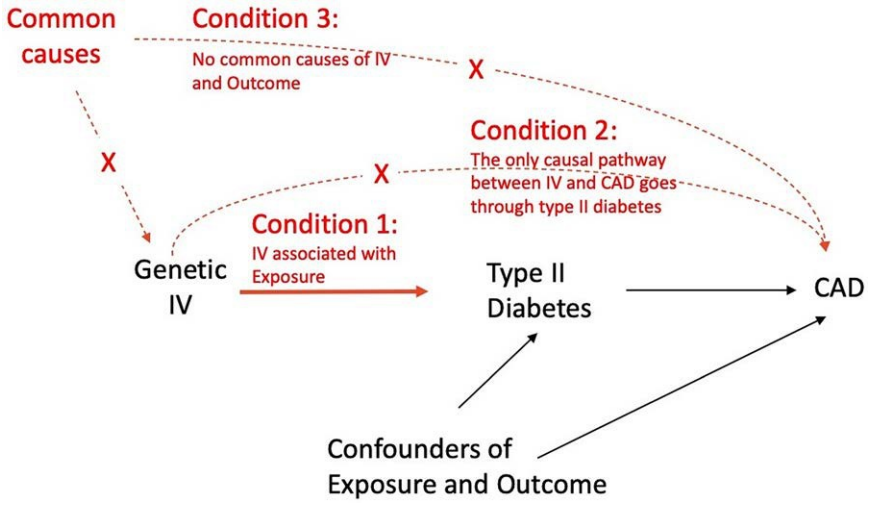

Figure 5.Violations of assumptions for Mendelian randomization analysis of type II diabetes and coronary artery disease (CAD).
IV indicates instrumental variable.

COMMON CAUSE FOR GENETIC VARIANTS AND DISEASE (CONDITION 3)

The third key assumption of Mendelian randomization analysis is that there is no common cause of the genetic variants and the disease—that is no confounding of the association between genetic variant and disease. Popu-lation stratification is one example of violation of this assumption. This occurs where population subgroups have different frequencies of the genetic variants under study, and differing risk of disease for genetic and envi-ronmental reasons that are unrelated to the genetic variants of interest.[52] For example, in a study of 4920 Native Americans of the Pima and Papago tribes, a very strong negative association was found between a par-ticular genetic variant for the Gm haplotype and type II diabetes (prevalence ratio=0.27 [95% CI, 0.18–0.40]).[53] However, the association disappeared when analysis was restricted to full-heritage Pima-Papago Americans. Rather than representing a genetic cause of T2DM, the genetic variant was actually found to be a marker for European ancestry. The authors concluded that it was most likely the presence of European genetic vari-ants and the concomitant decrease of Native American genetic variants that lowered the risk for diabetes, rath-er than the direct action of the genetic variant (genet-ic, environmental, and cultural differences between those with and without European ancestry are likely to have contributed to the observed differences in risk). Methods to deal with population stratification include restricting analysis to ethnically homogeneous groups and adjusting for ancestrally informative markers from genome-wide data. Other examples where the no com-mon cause condition may be violated are where there is linkage disequilibrium, unknown kinship in the study population, and where genetic variants (or by traits that are influenced by genetic variants) influence selection into both the study samples for associations between genetic variants and the risk factor and between genet-ic variants and the outcome.

TIME ZERO AND OTHER POTENTIAL SOURCES OF BIAS

An important concept when thinking about causality is that of time zero. In randomized trials, time zero is the time where treatment is assigned, eligibility criteria are met, and the outcome events of interest start to be counted. In Mendelian randomization studies, there is no clear time zero where these 3 events co-occur: treat-ment assignment is time of conception, eligibility crite-ria are some time after conception in childhood (need to survive long enough to be included in the study), and outcome events start being counted later in life. These restrictions to the study population after randomization are likely to bias estimates of causal associations, just

as postrandomization exclusions and loss to follow-up would in a randomized controlled trial.

Other sources of bias that may challenge the Mendelian randomization method include dynastic effects (potential confounders that are transmitted across generations) and assortative mating (choice of partner is not random and parental pairs are more alike than would be expected by chance). Family studies (eg, sibling pairs or parent-off-spring trios) may offer a potential solution to these issues and are an active area of research.[54] A number of other recent reviews in the cardiovascular field discuss further issues in Mendelian randomization studies.[55–57]

CONCLUSIONS

DAG may be used to explicitly define possible causal pathways under study to inform design of the Mendelian randomization study so that IV analysis may be used for causal inference. Well-designed Mendelian randomiza-tion studies are an important method for investigating causal effects of cardiovascular risk factors, such as type II diabetes, especially in situations where randomized trials may not yet exist and are not feasible. Although informative, these types of study require strong non-testable assumptions, and their results should not be considered definitive on their own. Recently published reporting guidelines that encourage authors to clearly state and assess the assumptions of the method,[58] may increase our confidence in the quality and reliability of results. By usefully contributing to the totality of evi-dence from several lines of enquiry, Mendelian random-ization studies may increase our certainty about the effectiveness (or otherwise) of interventions to reduce cardiovascular disease.

An example of a 2-sample Mendelian randomization analysis, using the software R, is provided in the supple-mentary material. Note that this analysis is for didac-tic purpose and the single-nucleotide polymorphisms used, although based on real data, were chosen in an ad-hoc manner.

ARTICLE INFORMATION

The Data Supplement is available at https://www.ahajournals.org/doi/suppl/10.1161/CIRCOUTCOMES.119.005623.

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