Original Research Article

Title: Bias from competing risk before recruitment in Mendelian Randomization studies of conditions with shared etiology

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Conflicts of interest: There is no conflict of interest

Sources of financial support: None

Data & Code: The data used here is publicly available, the code is available on request.

Acknowledgements: None

Abstract: 248

Word count: 2436

Figures: 2

Tables: 1

Supplementary Tables: 1
Abstract

Mendelian randomization, i.e., instrumental variable analysis with genetic instruments, is an increasingly popular and influential analytic technique that can foreshadow findings from randomized controlled trials quickly and cheaply even when no study measuring both exposure and outcome exists. Mendelian randomization studies can occasionally generate paradoxical findings different in direction from randomized controlled trial estimates. Despite robustness to confounding, Mendelian randomization studies are, like all observational studies, open to selection bias from sampling issues, such as selecting on exposure and outcome, or selecting on surviving until study onset, because of the gap between randomization (at conception) and recruitment, often decades later. What has been less considered is that biases due to selective survival on genetically predicted exposure can be compounded by selective survival on other diseases that share etiology with the outcome of interest, i.e., competing risk before recruitment. Many major causes of death, such as heart disease and stroke, share etiology. Here we show that, in the presence of survival on genetically predicted exposure, failure to account for competing risk of the outcome due to shared etiology can generate reversed Mendelian randomization estimates. We also explain when such selection bias is likely to occur. Mendelian randomization studies that do not take account of shared etiology of the outcome with survival likely have the greatest validity if the genetically predicted exposure does not cause death or when it does cause death the participants are recruited before many deaths have occurred from other diseases with which the outcome shares etiology.
Keywords: selection bias, competing risk, Mendelian randomization, instrumental variable analysis, shared etiology
Mendelian Randomization (MR), i.e., instrumental variable analysis with genetic instruments, is an increasingly popular and influential analytic technique [1, 2], which can be used to investigate causal effects even when no study including both exposure and outcome of interest exists. Invaluably, MR studies have provided estimates more consistent with results from randomized controlled trials (RCTs) than conventional observational studies, even foreshadowing the results of major trials [3]. MR studies are often presented as observational studies analogous to RCTs [4, 5] because they take advantage of the random assortment of genetic material at conception, while observational studies are open to biases from confounding and selection bias [6]. Instrumental variable analysis is described in health research as addressing confounding [7, 8], i.e., bias from common causes of exposure and outcome [6]. MR is currently described as “less likely to be affected by confounding or reverse causation than conventional observational studies” [2].

MR was originally thought to be less open to selection bias than conventional observation studies [9]. Selection bias is now increasingly widely recognized as a limitation of MR [10-20] which may violate the instrumental variable assumptions. Sources of potential selection bias in MR have been specifically identified as selecting an unrepresentative sample [10, 12, 13], attrition from an initially representative sample, such as a birth cohort [13], and selecting a sample strongly on surviving the exposure [11] or genotype of interest [16]. What has not explicitly been considered is selecting the sample on surviving the genotype/exposure of interest in the presence of competing risk of the outcome. MR studies are particularly vulnerable to sample selection on survival because of the time lag between randomization (at conception) and typical recruitment into studies of major diseases in middle- to old-age. MR studies also often concern major causes of death thought to share considerable etiology. For example, smoking, lipids, blood pressure and diabetes cause both ischemic heart disease (IHD) and ischemic stroke, with death from IHD typically occurring at younger ages than death from stroke [21]. As a result, a study of the association of lipids with stroke among the living will automatically select on surviving unhealthy lipids and on surviving competing risk of prior death from IHD due to smoking, blood pressure, diabetes and any
other shared etiology. Here, we consider how such situations relate to the instrumental variable assumptions, provide illustrative examples, explain both the relevance to paradoxical MR findings and to current areas of research, the implications for MR and show the insights they can generate.

Potential biasing pathways due to selective survival

Figure 1a shows the directed acyclic graph for MR illustrating the instrumental variable assumptions typically referred to as relevance, independence and exclusion-restriction. Relevance is explicitly indicated by the arrow from instrument to exposure. Independence is implicitly indicated by the lack of an arrow from confounders of exposure on outcome to instrument. Exclusion-restriction is implicitly indicated by the lack of arrows linking instrument to outcome, sometimes illustrated as no arrow from instrument to outcome indicating no pleiotropy [22-25] (Figure 1b). Figure 1c shows selection on survival of both instrument and common causes of the outcome \( U_2 \) [12, 19], which also violates the exclusion restriction assumption, particularly when stated as “every unblocked path connecting instrument and outcome must contain an arrow pointing into the exposure” [26]. Figure 1d explicitly shows survival on instrument, and another disease \( Y_2 \) sharing etiology \( U_2 \) with the outcome \( Y_1 \). Figure 1e shows the exclusion restriction assumption with both no pleiotropy and no selection bias from competing risk made explicit.

Notably, Figures 1c and 1d are very similar in structure to a well-known example of selection bias which occurs when conditioning on an intermediate reverses the direction of effect: the “birth weight” paradox [27]. In the birth weight paradox, the positive association of maternal smoking with infant death becomes inverse after adjusting for birth weight, likely because birth weight is affected by maternal smoking, and by a common cause of birth weight and infant death, i.e., infant defects, which was not included in the analysis [27]. In the birth weight paradox adjusting for all common causes of birth weight and survival, i.e. birth defects, should remove the bias [27] because it blocks the path from survival to outcome.
Common study designs, such as cross-sectional, case-control or cohort, usually recruit from those currently alive. Even a population representative sample has inevitably suffered attrition from death before recruitment relative to the original underlying birth cohorts. In such studies selection bias from competing risk before recruitment would be particularly marked when assessing the effect of a harmful exposure on an outcome that shares etiology with other diseases that commonly cause death at earlier ages than the outcome of interest (Figure 1d). As such, when the exposure (and instrument) have no effect on the outcome bias under the null could occur, null estimates may be the result of bias, and reversals may occur. Paradoxical reversals of effects in observational studies of older people or sick people are well known as examples of selection bias, such as smoking being inversely associated with dementia [28], which has also occurred in an MR studies [29]. MR studies also consistently suggest no effect of adiposity on stroke [30], which could be an overlooked etiological difference between IHD and stroke or could be due to bias from inadvertently selecting the sample on surviving genetically predicted adiposity and IHD because of the many shared causes of IHD and stroke (Figure 2a).

**Illustrative example**

Statins and PCSK9 inhibitors are well-established interventions for cardiovascular disease, which reduce low density lipoprotein (LDL)-cholesterol, IHD [31-33], stroke [31-33] and atrial fibrillation (AF) [34]. IHD, stroke and AF also share major causes independent of LDL-cholesterol, such as blood pressure [35, 36]. Death from IHD typically occurs at earlier ages than death from stroke in Western populations [21]. AF may also be a consequence of IHD. Statins also appear to have a greater effect on overall survival than PCSK9 inhibitors [32, 34, 37, 38]. As such, given Figure 2b, greater bias would be expected when using MR to assess effects of harmful exposures on stroke and AF than on IHD, for studies with participants recruited at older ages and possibly more for genetically predicted statins than PCSK9 inhibitors.

We obtained genetically predicted LDL based on well-established genetic variants predicting statins (rs12916, rs17238484, rs5909, rs2303152, rs10066707 and rs2006760) and PCSK9 inhibitors
(rs11206510, rs2479409, rs2149041, rs2479394, rs10888897, rs7552841 and rs562556) [39]. We applied these variants to major genome wide association studies (GWAS) in people largely of European descent of IHD (CARDIoGRAMplusC4D 1000 Genomes) [40], ischemic stroke (MEGASTROKE) [41] and AF (Nielsen et al) [42] and to the UK Biobank summary statistics for IHD and ischemic stroke [43], but not AF because the GWAS includes relevant data from the UK Biobank [42]. Appendix Table 1 gives descriptive information about these GWAS. We obtained inverse variance weighted MR estimates with multiplicative random effects taking into account any correlations between genetic variants for European populations obtained from LD-Link (https://ldlink.nci.nih.gov) using the Mendelianrandomization R package.

Table 1 shows the MR associations of genetically predicted lower LDL-cholesterol, based on statin and PCSK9 inhibitor variants, with each disease using different outcome GWAS. As expected, the MR estimates show that genetically predicted lower LDL-cholesterol, based on statin or PCSK9 inhibitor genetic variants, reduced IHD, albeit with a slightly less marked effect for statin genetic variants on IHD in the UK Biobank. LDL-cholesterol lowering, based on statin or PCSK9 inhibitor genetic variants, was not associated with a lower risk of stroke. LDL-cholesterol lowering, based on statin genetic variants, had an association in a positive direction with AF while LDL-cholesterol lowering, based on PCSK9 inhibitor genetic variants, had an association in the opposite direction with AF. The contradictory results for stroke and AF compared to IHD could be due to differences in the underlying populations, but we replicated the findings for IHD and stroke in the UK Biobank. They could also be chance findings, but we have shown that stroke GWAS are open to systematic type II error from competing risk [44]. More parsimoniously, selection bias invalidating the exclusion restriction assumption of instrumental variable analysis as shown in Figure 2b could be at play. Statins and PCSK9 inhibitors affect survival but death from IHD between randomization and recruitment precludes seeing their full effect on stroke and AF. Correspondingly, from the limited information about the underlying GWAS available, the AF cases appear to be older than the stroke cases who were older than the IHD cases (Supplementary Table 1).
Paradox explained

A previous MR study has similarly shown LDL-cholesterol lowering PCSK9 inhibitor genetic variants causally associated with IHD but not with stroke [45], and suggested the study showed the limits of MR [45]. Figure 2b suggests this anomalous finding for stroke may be the result of selection into the stroke GWAS dependent on surviving the harmful effects of LDL-cholesterol genetic variants and any other factors, such as blood pressure, obesity, diabetes and smoking, causing IHD and stroke without accounting for them. As such, the study in question does not show the limits to MR per se [45], but specifically an addressable violation of the instrumental variable assumption of exclusion-restriction in that MR study.

Insights obtained

Previous MR studies have suggested that smoking and hypertension protect against Alzheimer’s disease (AD) [29]. On the one hand, these observations could reflect true causal relations. On the other hand, given smoking and hypertension adversely affect survival, these anomalous associations could be the result of shared etiology with other common conditions, such as IHD, that typically results in death before the average age of onset AD, and thereby preclude recruitment into the relevant studies. ApoE is a very well-established cause of AD [29]. ApoE also causing IHD would explain the anomalous inverse associations of smoking and hypertension with AD, as well as shedding new light on IHD, as shown in Figure 2c.

Potential solutions

Many MR studies, apart from those based on birth cohorts, have a substantial time lag between conception and recruitment, and may consider conditions which share etiology with common diseases that can cause death and occur earlier in life. For example, several relatively late onset cardiovascular conditions, such as stroke, atrial fibrillation, heart failure and aneurysm are thought to share etiology with a common earlier onset cardiovascular condition, IHD. To what extent, selection bias due to surviving the genetically predicted exposure and competing risk from shared etiology occurs for other major causes of death such...
as cancer and respiratory is less clear, although smoking, obesity, lack of physical activity and diet are common causes of many chronic diseases.

The conceptually simplest way to address bias from surviving to recruitment on genetically predicted exposure in the presence of competing risk of the outcome is to use inverse probability weights of such survival, but these are often unknown. The alternative way to block the path from instrument to outcome is to adjust for all factors underlying the shared etiology of outcome and competing risk. However, that these factors are not comprehensively known is often the motivation for an MR study. Moreover, such comprehensive information is not usually collected and is not yet available even for the major genotyped cohorts, such as the UK Biobank or the China Kadoorie Biobank, although multivariate MR might provide a partial solution to account for known shared etiology. More generally, accounting for such factors is also seen as a barrier to obtaining precise estimates in genetic studies [46], and an active area of research in MR studies [2], for which we have provided some clarification. However, it may be unclear exactly how to adjust as some common causes of the outcome may also be consequences of the exposure thereby generating pleiotropic effects.

Here, we have made a conceptual and empirical argument for accounting for shared etiology in MR studies, rather than using simulation, because the key issue is to recognize when biases in MR may occur, which is addressed not by simulation but by external contextual knowledge about the research question. For example, MR phenoome wide association studies of the effect of one exposure on many outcomes are unlikely to be valid unless the exposure is non-fatal or consideration is given to accounting for shared etiology generating outcome specific competing risk.
Checking the underlying genetic studies used in MR for validity directly is difficult because few genotypes are known to act via well-established physiological pathways with known effects, but other approaches are possible. First, replication using different genetic studies is increasingly possible. However replication studies could all be subject to the same biases [47]. Second, associations that change with recruitment age are indicative of a harmful effect and potential bias [48, 49], with the associations in younger people having the greatest validity [15] because selective survival and competing risk prior to recruitment will usually be greater with age, as mortality rates for most conditions increase with age. However, this is a heuristic to identify flawed studies, rather than a solution; although perhaps preferable to assuming a null MR estimate has more reliability than any other value [50]. Third, sensitivity analyses could perhaps be used to quantify the level of selection bias [51-54] and competing risk. Finally, the issue here of obtaining valid MR estimates in the presence of selective survival on exposure and competing risk of the outcome is conceptually similar to the issue of obtaining valid genetic estimates in other studies of survivors, i.e., patients. However, the current solution for obtaining valid estimates in genetic studies of patients relies on the assumption that the factors causing disease and disease progression differ [55].

Conclusion

Here, we have shown theoretically and empirically that MR studies are open to selection bias arising from selective survival on genetically instrumented exposure, particularly for diseases with shared etiology, when other causes of survival and outcome exist, i.e. competing risk before recruitment. Moreover, we have to some extent addressed the active area of research as to whether MR studies should adjust for other traits [2], and explained how this issue relates to the assumptions of instrumental variable analysis. Bias from such selection bias is likely to be least evident for MR studies of harmless exposures recruited shortly after genetic randomization with no competing risk, i.e., studies using birth cohorts with minimal attrition. Conversely, such bias is likely to be most evident for MR studies recruited at older ages examining the effect of a harmful exposure on an outcome subject to competing risk from shared etiology.
with other common conditions that occur earlier in life. More methods of obtaining valid MR estimates, when the exclusion restriction is invalidated by selection bias stemming from competing risk, are required.
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Table 1: Effect of LDL-cholesterol lowering (1mmol/L) by genetically predicted statin and PCSK9 inhibitor [39] on IHD, stroke and AF using Mendelian randomization applied to the CARDIoGRAMplusC4D 1000 Genomes based GWAS of IHD [40], UK Biobank summary statistics from SAIGE for IHD and stroke [43], MEGASTROKE [41] for stroke and a study by Nielsen et al [42] for AF and the effects of statins and PCSK9 inhibitors on the same outcomes from meta-analysis of RCTs [32, 34, 37, 38]

| Disease                        | Source of genetic associations                  | Estimates from Mendelian Randomization of genetically predicted LDL lowering by | Estimates from Meta-analysis of RCTs [32, 34, 37, 38] with lipid lowering by |
|-------------------------------|------------------------------------------------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------|
|                               |                                                 | Statins 95% CI                                                                   | PCSK9 inhibitors 95% CI                                                  | Statins 95% CI                                                                   | PCSK9 inhibitors 95% CI |
| Ischemic heart disease        | CARDIoGRAMplusC4D 1000 Genomes                 | 0.59 0.44 to 0.81                                                               | 0.55 0.35 to 0.87                                                          | 0.69 0.61 to 0.77                                                               | 0.72 0.64 to 0.81 |
|                               | UK Biobank (SAIGE)                              | 0.69 0.48 to 0.996                                                             | 0.56 0.44 to 0.70                                                          |                                                                                |                       |
| All ischemic stroke           | MEGASTROKE                                      | 1.01 0.72 to 1.41                                                               | 1.08 0.97 to 1.22                                                          | 0.71 0.62 to 0.82                                                               | 0.80 0.67 to 0.96 |
|                               | UK Biobank (SAIGE)                              | 1.41 0.81 to 2.49                                                               | 0.85 0.57 to 1.28                                                          |                                                                                |                       |
| Atrial fibrillation           | Nielsen et al                                   | 1.14 0.92 to 1.42                                                               | 0.85 0.71 to 1.01                                                          | 0.47 0.30 to 0.75                                                               | na                     |

Supplementary table 1: Study details for the GWAS of IHD, stroke and AF

| Study                        | Phenotype (Phewas code) | Cases | Non-cases | Mean age of cases | Phenotype definition                                                                 | Adjusted for (non-genetic) |
|------------------------------|-------------------------|-------|-----------|-------------------|--------------------------------------------------------------------------------------|---------------------------|
| Cardiogram 1000 genomes GWAS [40] | Ischemic heart disease | 60,801 | 123,504 | n/a, possibly ~58 years | "Case status was defined by an inclusive CAD diagnosis (e.g. myocardial infarction (MI), acute coronary syndrome, chronic stable angina, or coronary stenosis >50%)" | Study-specific covariates (not age or sex) |
| UK biobank SAIGE [43]        | CAD (411) Stroke (433)  | 31,355 | 8,742     | 377,103           | Phewas code based on self-report, hospital episodes and death                         | Sex, birth year, and principal components 1 to 4 |
|                              | AF (427.2)              | 14,820 | 399,017   | 380,919           |                                                                                       |                           |
| MEGASTROKE [41]              | All ischemic stroke     | 60,341 | 454,450   | ~69 years         | Several different definitions used                                                   | Minimum of age and sex    |
| AF [42]                      | Atrial fibrillation     | 60,620 | 970,216   | ~74 years         | Usually based on ICD-9 427.3 and ICD-10 148                                         | Minimum of age (birth year) and sex |
Figure 1: Directed acyclic graphs with instrument (Z), outcome (Y), exposure (X), confounders (U) and survival (S), where a box indicates selection, for a) a valid Mendelian randomization study, and b) a Mendelian randomization study with an invalid instrument through violation of the exclusion-restriction assumption via pleiotropy, c) a Mendelian randomization study with an invalid instrument through violation of the exclusion-restriction assumption via survival on instrument and shared etiology with the outcome (U<sub>2</sub>), d) a Mendelian randomization study with an invalid instrument through violation of the exclusion restriction assumption via survival (S), competing risk of another disease (Y<sub>2</sub>) and shared causes (U<sub>2</sub>) with (Y<sub>2</sub>) and the outcome (Y) and e) a Mendelian randomization illustrating both conditions which have to be met to satisfy the exclusion restriction assumption.

Figure 2: Directed acyclic graphs showing examples where selection bias could occur because of selection on survival (S), indicated by a box, on the instrument (GV) and on competing risk of ischemic heart disease (IHD) which shares causes with the outcome. U<sub>1</sub> indicates confounders of exposure on outcome. AD indicates Alzheimer’s disease.
