Original Research Article

Title: Survival bias and competing risk can severely bias Mendelian Randomization studies of specific conditions

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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: 223

Word count: 2229

Figures: 1

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 that differ from estimates obtained from randomized controls trials, potentially for important, yet to be discovered, etiological reasons. However, bias is always a possibility. Here we demonstrate, with directed acyclic graphs and real-life examples, that Mendelian randomization estimates may be open to quite severe bias because of deaths occurring between randomization (at conception) and recruitment years later. Deaths from the genetically predicted exposure (survival bias) and when such deaths occur deaths from other causes of the outcome (competing risk) both contribute. Using a graphical definition of the condition for a valid instrument as "*every unblocked path connecting instrument and outcome must contain an arrow pointing into the exposure"* draws attention to this bias in Mendelian randomization studies as a violation of the instrumental variable assumptions. Mendelian randomization studies 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 such deaths have occurred and before many deaths have occurred due to other causes of the outcome.

Keywords: selection bias, competing risk, Mendelian randomization, instrumental variable analysis

Mendelian Randomization (MR), i.e., instrumental variable analysis with genetic instruments, is an increasingly popular and influential analytic technique [1, 2], which can even 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, when 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 providing unconfounded estimates [2].

MR is also thought to be fairly robust to selection bias [9]. Nevertheless, selection bias in MR has been identified as potentially arising from sample selection, such as selecting unrepresentative samples [10, 11], particularly when using one sample for an MR study [12], selecting on exposure or outcome and confounders of exposure on outcome [11] or selecting on exposure and outcome [13]. However, the full practical implications for MR estimates of a time lag between randomization at conception and study recruitment often in late adulthood [14] have not been fully considered. Although several scenarios relevant to selection bias have been addressed, such as selective survival on exposure [15, 16], on exposure and outcome [17], on exposure and other causes of the outcome [18-20] or on instrument and other causes of the outcome [12, 19], i.e. competing risk. Extensive attention has been given in MR to the possibility that the genetic instruments might be invalidated by acting directly on the outcome other than via the exposure due to pleiotropic genetic effects, i.e., the same genetic instruments acting via a range of phenotypes. Many analytic techniques have been developed to address this eventually, such as MR-Egger [21], MR-PRESSO [22] and mode or median based estimates [23, 24], often focusing on identifying heterogeneity among genetic instruments. Less attention has been given to the full implications of the genetic instruments being linked to the outcome in other ways [12, 19] that invalidate the genetic

instruments, such as biasing pathways arising from selective survival on instrument in the presence of competing risk of the outcome. Here, we consider how such pathways relate to the instrumental variable assumptions, provide illustrative examples, show how they might explain paradoxical MR findings, explain their implications for MR and provide some suggestions.

Potential biasing pathways from instrument to outcome 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 specifically as no arrow from instrument to outcome [21-24], as in Figure 1b, which shows an invalid instrument due to pleiotropy. However, violation of the exclusion-restriction assumption can occur in other ways, as made clear by the graphical definition of a valid instrumental variable: *"every unblocked path connecting instrument and outcome must contain an arrow pointing into the exposure"* [25]. Figure 1c shows an unblocked pathway from instrument to outcome, due to selection on instrument and outcome. Figures 1d and 1e show survival on both instrument and common causes of the outcome (U_2) [12, 19]. The time lag between randomization (at conception) and typical recruitment into genetic studies of major diseases in middle- to old-age means some MR studies may inevitably recruit on surviving both the genetic instrument(s) and competing risk of the outcome. As such, violations of the exclusion restriction assumption due to such sample selection (Figures 1d and 1e) can result in invalid instruments potentially biasing MR estimates in contrast to previously discussed situations where MR with potentially valid instruments may be open to selection bias (Figures 1f, 1g and 1h) [11, 13, 16, 18-20].

Notably, figures 1d and 1e 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

[26]. 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 [26]. In the birth weight paradox adjusting for all common causes of birth weight and survival, i.e. birth defects, should remove the bias [26], as long as no effect measure modification exists.

Common study designs, such as cross-sectional, case-control or cohort, usually recruit from those currently alive, which even if the sample is population representative, may not encompass selection from the original underlying birth cohorts, and may also be open to competing risk when considering a particular disease rather than overall survival. As such, even population representative studies are open to selective survival before recruitment, particularly in the presence of competing risk. In such studies the level of bias will be least if the instrument has no effect on survival, or when it does affect survival if no other risk factors affect survival to recruitment and outcome (Figure 1c), for example when studying a disease of old age in young people. However, bias would be particularly marked for an outcome that has risk factors that typically cause death from other diseases at earlier ages, i.e., competing risk (Figures 1d and 1e), particularly if the participants are recruited at an advanced age. Paradoxical reversals of effects in observational studies of older people or sick people are well known as examples of selection bias, such as cigarette use inversely associated with dementia [27]. MR studies are as open to this problem as any other observational study. For example, MR studies consistently suggest no effect of adiposity on stroke [28], which could be an overlooked etiological difference between IHD and stroke or could be bias.

Illustrative example

Statins and PCSK9 inhibitors are well-established interventions for cardiovascular disease, which reduce low density lipoprotein (LDL)-cholesterol, IHD [29-31], stroke [29-31] and atrial fibrillation (AF) [32]. IHD, stroke and AF also share major causes independent of LDL-cholesterol, such as blood pressure [33, 34]. Death from IHD typically occurs at earlier ages than death from stroke in Western populations [35].

AF may also be a consequence of IHD. Statins also appear to have a greater effect on overall survival than PCSK9 inhibitors [30, 32, 36, 37]. As such, bearing in mind Figures 1d and 1e, 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) [38]. We applied these variants to major genome wide association studies (GWAS) in people largely of European descent of IHD (CARDIoGRAMplusC4D 1000 Genomes) [39], ischemic stroke (MEGASTROKE) [40] and AF (Nielsen et al) [41] and to the UK Biobank summary statistics for IHD and ischemic stroke [42], but not AF because the GWAS includes relevant data from the UK Biobank [41]. 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-cholestrol, 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 and other GWAS are open to systematic bias from competing risk [43]. More parsimoniously, selection bias invalidating an assumption of instrumental variable analysis as shown in Figures 1d and 1e 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. 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 [44], and suggested the study showed the limits of MR [44]. Figures 1d and 1e suggest 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 or smoking, causing survival and stroke without adjusting for all these common causes of survival and stroke. As such, the study in question does not show the limits to MR per see [44], but specifically a violation of the instrumental variable assumption of exclusion-restriction in that MR study.

Potential solutions

Many genetic studies, apart from those based on birth cohorts, have a substantial time lag between conception and recruitment, and often consider specific conditions, which can bias MR studies. As such, consideration of the "exclusion-restriction" assumption in MR should not only consider pleiotropic effects of the genetic instruments on outcome acting other than via the exposure,[45] but also any unblocked pathways linking the genetic instrument with the outcome without an arrow into the exposure (Figures 1c, d and e).

Clearly, MR studies depend on the validity of the underlying genetic studies, which may be similarly biased by survival and competing risk before recruitment [43]. Possibly with a greater risk of bias for two sample MR where selection bias in the instrument outcome associations is less likely to be cancelled out by similar biases in the instrument exposure associations. Checking genetic studies 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 [46]. Second, use of control exposures and outcomes in MR studies, subject to the same bias, would be helpful [47], but few causal effects of genotypes are known. Third, checking that MR studies have effects consistent in direction with evidence from RCTs would give credence. Fourth, associations that change with recruitment age are indicative of a harmful effect [48, 49], with the associations in younger people having greatest validity [15], because selective survival 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, but perhaps better than assuming a null MR estimate has more reliability than any other value [50]. Fifth, sensitivity analyses could perhaps be used to quantify the level of selection bias [51-54]. Finally, the issue here of obtaining valid MR estimates in the presence of selective survival 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 are different [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 when other causes of survival and outcome exist. This bias arising from violating an assumption of instrumental variable analysis 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 considering survival as the outcome. Conversely, such

bias is likely to be most evident for MR studies recruited at older ages examining the effect of a harmful exposure when many other risk factors for survival and outcome exist. Consideration of an overlooked assumption of instrumental variable analysis, i.e., for an instrument to be valid *"every unblocked path connecting instrument and outcome must contain an arrow pointing into the exposure"* [25], possibly as part of evaluation of the exclusion restriction assumption, may help identify this bias in MR studies. More methods of obtaining valid MR estimates when using invalid instruments are required.

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Table 1: Effect of LDL-cholesterol lowering (1mmol/L) by genetically predicted statin and PCSK9 inhibitor [38] on IHD, stroke and AF using Mendelian randomization applied to the CARDIoGRAMplusC4D 1000 Genomes based GWAS of IHD [39], UK Biobank summary statistics from SAIGE for IHD and stroke [42], MEGASTROKE [40] for stroke and a study by Nielsen et al [41] for AF and the effects of statins and PCSK9 inhibitors on the same outcomes from meta-analysis of RCTs [30, 32, 36, 37]

		Estimates from							
		Mendelian Randomization of genetically predicted LDL lowering by				Meta-analysis of RCTs [30, 32, 36, 37] with lipid lowering by			
		Statins		PCSK9 inhibitors		Statins		PCSK9 inhibitors	
Disease	Source of genetic associations	OR	95% CI	OR	95% CI	OR	95% CI	OR	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 el 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 [39]	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 [42]	CAD (411) Stroke (433) AF (427.2)	31,355 8,742 14,820	377,103 399,017 380,919	n/a	Phewas code based on self-report, hospital episodes and death	Sex, birth year, and principal components 1 to 4
MEGASTROKE [40]	All ischemic stroke	60,341	454,450	~69 years	Several different definitions used	Minimum of age and sex
AF [41]	Atrial fibrillation	60,620	970,216	~74 years	Usually based on ICD-9 427.3 and ICD-10 I48	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, d) and e) Mendelian randomization studies with invalid instruments through violation of the exclusion restriction assumption via survival and competing risk, and f), g) and h) Mendelian randomization studies potentially open to selection bias.