Supplemental Appendix

Using negative control populations to assess unmeasured confounding and direct effects

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# eText

## eText 1: Formal derivations

### Proof proposition 1

If the association between the exposure and the outcome is null in the negative control population, then

for all

 for all .

Thus,

.

Under the additional assumption that ,

### No direct effect and no unmeasured confounding in V=1 imply no A-Y association in V=1

#### Relying on A4a

Therefore, under no direct effect

 w.p.1 for all

and A4a, there is no average causal effect in the negative control population

.

If we further assume no unmeasured confounding, w.p.1 for all a, we have that

.

That is, unexposed and exposed individuals in the negative control population have the same expected outcome.

#### Relying on A4b

Therefore, under the assumption of no direct effect,

 for all ,

there is no average causal effect in the negative control population

.

As before, when there is no confounding, then the unexposed and exposed individuals in the negative control population have the same expected outcome.

## eText 2: Collider bias and definitions of negative control populations

Suppose that we are interested in verifying the exclusion-restriction assumption in a Mendelian randomization study. The instrument is a genetic variant, , associated with alcohol consumption, . Let the health-related outcome be denoted by . We are interested in using as an instrument to quantify the effect of on because we are worried about the presence of an unmeasured common cause of alcohol consumption and the outcome.

Informally, for the instrumental variable method to be valid, we need to ensure that the genetic variant has no effect on the outcome other than through alcohol consumption, which is an exclusion-restriction assumption; there cannot be a direct effect of on outside of . This set-up mirrors the one from Topiwala et al. where the outcome is telomere length.1 The data generation process is represented in the DAG in eFigure 1a.

Topiwala et al. used a negative control to rule out the presence of a direct causal effect of the gene, outside of alcohol consumption. They defined the negative control population as individuals who do not drink alcohol.1 This choice seems to be motivated by the idea that, among non-drinkers, the genetic variant does not determine the level of alcohol consumption. The idea that non-drinkers represent a suitable negative control population also appear elsewhere2 and is compatible with Davies et al., who stated that a negative control population “has a similar confounding structure as the population of interest but was not exposed to the treatment of interest.”3 In the Mendelian randomization setting, the term exposure is used to refer to the mediator, rather than , as we do in the main text.

However, restricting the analysis to non-drinkers means conditioning on a level of the variable (e.g., zero alcohol consumption,). This closes the path , but conditioning on also opens, for example, the previously closed path . This is because is a collider on the path . Thus, among non-drinkers we expect to observe an association between the genetic variant and the outcome, regardless of the presence of a direct effect of on . Therefore, the group of non-drinkers does not generally represent a useful negative control population for this research question. In particular, the investigators could conclude that the exclusion-restriction assumption is violated, even when it is actually satisfied. We emphasize that in the described scenario we are imagining that no confounding exists between and . Therefore, non-drinkers could be perceived to satisfy the informal definition of Davies et al. that requires a “similar confounding structure.” The issue is that non-drinkers do not satisfy Assumption 4. They merely represent a subgroup (one level of ) for whom there would be no association between and if no common causes exist between and , and the exclusion restriction holds.

When discussing a similar example, Richardson and Tchetgen Tchetgen4 defined a negative control population as “a population in which alcohol consumption is absent.” While this definition is ostensibly similar to the one from Davies et al., it describes a different causal structure; they do not define the negative control population as a group of individuals who are not exposed to alcohol, but as a group of individuals who have characteristics such that they deterministically do not consume alcohol (irrespective of the genetic profile4). This definition agrees with Assumption 4a and with the rationale of other researchers.2,5 For example, may represent women in a specific population where they abstain from alcohol for cultural reasons.2,5 In this population of women, we expect no effect of the genetic variant on the alcohol consumption level (eFigure 1c). Here, the strong positivity assumption from the main text is violated but our main results are still valid. Indeed, it is sufficient that

 if and only if for all

which holds because in this example we have for all u and all a.

de Leeuw et al. also described the problem of collider stratification bias in analyses based on negative control populations.6 They argued that individuals who do not drink alcohol due to cultural reasons constitute a valid negative control population, as in this group there is a “constraint” on the value of the exposure (). They further described the difference between the setting where the exposure is “constrained” (fixed) in a subgroup, and the setting where individuals are “selected” because of their exposure value, where only the selection results in collider stratification bias.6 We emphasize, however, that both de Leeuw et al.6 and Davies et al.3 used a narrow definition of negative control population. They defined a negative control population as a population where the variable is (causally) constrained to one value.3,6 This definition only covers settings where the relationship between and is deterministic. We showed in the main text that weaker definitions of negative control population are valid.

## eText 3: BEST-MSU trial details

The BEST-MSU trial was a prospective multicenter study that ran between August 2014 and August 2020 in seven cities in the USA and aimed at assessing the effect of Mobile Stroke Unit additional dispatch on clinical outcomes.7 In every participating site, MSU was dispatched together with a conventional ambulance on alternate weeks for potential stroke patients within 4.5 hours after the onset of symptoms.7 The enrollment in the trial was determined upon arrival on the scene based on stroke suspicion, disability, time from onset, and no obvious contraindication for tissue plasminogen activator (t-PA).7 The eligibility for the main analysis was instead determined from a blinded vascular neurologist a posteriori.7

## References

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2. van de Luitgaarden IAT, van Oort S, Bouman EJ, et al. Alcohol consumption in relation to cardiovascular diseases and mortality: a systematic review of Mendelian randomization studies. *Eur J Epidemiol*. 2022;37(7):655-669.

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5. Peng M, Zhang J, Zeng T, et al. Alcohol consumption and diabetes risk in a Chinese population: a Mendelian randomization analysis. *Addiction*. 2019;114(3):436-449.

6. de Leeuw C, Savage J, Bucur IG, Heskes T, Posthuma D. Understanding the assumptions underlying Mendelian randomization. *Eur J Hum Genet*. 2022;30(6):653-660.

7. Grotta JC, Yamal JM, Parker SA, et al. Prospective, multicenter, controlled trial of mobile stroke units. *N Engl J Med*. 2021;385(11):971-981.

# eFigures

## eFigure 1: DAG with randomized exposure



**eFigure 1. Panel (a) shows a Directed Acyclic Graph representing causal relationships between the exposure (A), the mediator (M), the outcome (Y), the unmeasured variable (U) and the variable indicating the negative control population (V). Panel (b) and (c) show Directed Acyclic Graphs conditional on V=1 in two possible settings where the exposure has no effect on the outcome through the mediator.**

# eAppendix

## eAppendix 1: R code

# load packages

library(tidyverse)

library(arsenal)

# import values from table S4 of Grotta et al. 2021

tableS4 <- data.frame(

 mrs=rep(c(0:6,NA),times=4),

 mrs\_uw=rep(c(1,0.91,0.74,0.65,0.19,0.03,0,NA),times=4),

 enrollment=rep(c("ischemic","all"),each=16),

 group=rep(c("msu","ems"),each=8,times=2),

 freq=c(126,123,82,109,124,68,19,0,

 38,91,71,78,92,64,18,0,

 153,157,112,134,178,115,36,1,

 51,117,84,105,143,99,30,0))

# derive counts for the non-ischemic group

list\_tableS4 <- tableS4 %>% group\_by(enrollment) %>% group\_split()

dat\_s <- bind\_rows(list\_tableS4[[2]],

 list\_tableS4[[1]] %>% mutate(enrollment="other", freq=list\_tableS4[[1]]$freq-list\_tableS4[[2]]$freq))

# use data in aggregated form to create the dataset

dat\_l <- dat\_s %>% uncount(freq)

dat\_l$group <- factor(dat\_l$group, levels=c("msu","ems"),ordered = T)

# descriptive statistics (Table 1)

tableby(interaction(group,enrollment) ~ factor(mrs) + mrs\_uw, data=dat\_l, numeric.stats=c("Nmiss","meansd","median","q1q3")) %>% summary(test=FALSE, total=TRUE, digits=3, digits.pct=0)

## Analysis

# exclude the individual with missing functional outcome

dat\_l <- drop\_na(dat\_l)

# association in the negative control population

t.test(mrs\_uw ~ group, data=dat\_l %>% filter(enrollment=="other"))

wilcox.test(mrs\_uw ~ group, data=dat\_l %>% filter(enrollment=="other"))

# marginal association

t.test(mrs\_uw ~ group, data=dat\_l)

wilcox.test(mrs\_uw ~ group, data=dat\_l)