# Example R and Stata code for a Mendelian randomization analysis

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# Example R and Stata code to perform a multivariable Mendelian randomization (MVMR) analysis

### R code

• Read in the data

```
suppressPackageStartupMessages({
   library(tidyverse)
   library(haven)
   library(remotes)
})
dat <- read_dta("https://raw.github.com/remlapmot/mrrobust/master/dodata.dta")
dat <- dat %>% filter(ldlcp2 < 1e-8)</pre>
```

#### Example code using the MendelianRandomization package

• Install the package and load it into memory

### library(MendelianRandomization)

• Convert our data frame to the required class

```
datfmt <- mr_mvinput(
    bx = as.matrix(cbind(dat$ldlcbeta, dat$hdlcbeta, dat$tgbeta)),
    bxse = as.matrix(cbind(dat$ldlcse, dat$hdlcse, dat$tgse)),
    by = dat$chdbeta,
    byse = dat$chdse,
    exposure = "exposure",
    outcome = "outcome",
    snps = "snp",
    effect_allele = dat$a1,
    other_allele = dat$a2,
    eaf = NA
)</pre>
```

• Fit an MVMR/MVIVW model

```
mvivwfit <- mr_mvivw(datfmt)
mvivwfit

##
## Multivariable inverse-variance weighted method
## (variants uncorrelated, random-effect model)
##</pre>
```

```
## Number of Variants: 73
##
## -----
    Exposure Estimate Std Error 95% CI
##
                                     p-value
## exposure_1 0.429 0.061 0.309, 0.548 0.000
## exposure 2 -0.194
                     0.131 -0.451, 0.062 0.138
                    0.123 -0.016, 0.468 0.067
## exposure 3 0.226
## -----
## Residual standard error = 1.490
## Heterogeneity test statistic = 155.3766 on 70 degrees of freedom, (p-value = 0.0000)
  • Fit an MVMR-Egger model
mvmreggerfit <- mr_mvegger(datfmt)</pre>
mvmreggerfit
##
## Multivariable MR-Egger method
## (variants uncorrelated, random-effect model)
##
## Orientated to exposure : 1
## Number of Variants : 73
                                         p-value
##
     Exposure Estimate Std Error 95% CI
##
   exposure 1 0.567 0.100 0.371, 0.764 0.000
##
    exposure_2 -0.136 0.133 -0.398, 0.125
                                          0.306
             0.274
                     0.125 0.030, 0.518
##
   exposure 3
                                          0.028
## (intercept) -0.009 0.005 -0.020, 0.001 0.084
## Residual standard error = 1.469
```

#### Example code using the MVMR and RMVMR packages

• Install the package and load it into memory

```
if (!requireNamespace("MVMR", quietly = TRUE)) {
  remotes::install_github("WSpiller/MVMR")
}
library(MVMR)
```

## Heterogeneity test statistic = 148.9290 on 69 degrees of freedom, (p-value = 0.0000)

• Create a data object of the required structure

```
r_input <- format_mvmr(
   BXGs = dat[,c("ldlcbeta","hdlcbeta","tgbeta")],
   BYG = dat$chdbeta,
   seBXGs = dat[,c("ldlcse","hdlcse","tgse")],
   seBYG = dat$chdse,
   RSID = dat$rsid
)</pre>
```

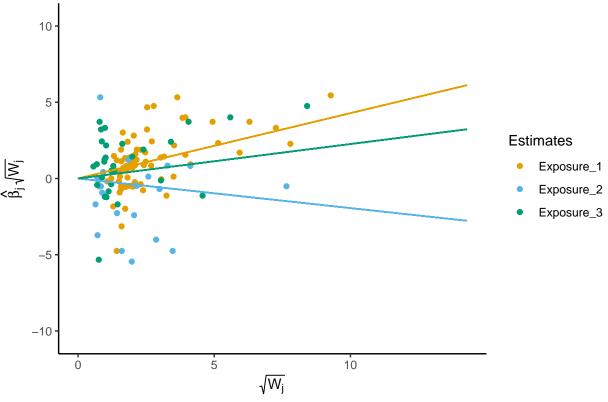
• Fit an MVMR model

```
mvmrfit <- ivw_mvmr(r_input)

##
## Multivariable MR
##</pre>
```

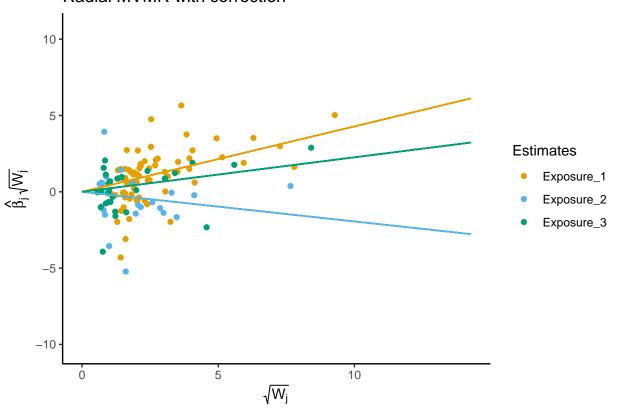
```
##
               Estimate Std. Error
                                    t value
## exposure1 0.4286200 0.0609661 7.030464 1.099077e-09
## exposure2 -0.1941989 0.1308289 -1.484372 1.421994e-01
## exposure3 0.2260456 0.1232828 1.833554 7.097168e-02
## Residual standard error: 1.49 on 70 degrees of freedom
  • Conditional F-statistics for instrument strength (Sanderson, Spiller, and Bowden 2021)
strength_mvmr(r_input)
## Warning in strength_mvmr(r_input): Covariance between effect of genetic variants on each exposure
## not specified. Fixing covariance at 0.
## Conditional F-statistics for instrument strength
##
##
               exposure1 exposure2 exposure3
## F-statistic 126.7447 35.29937 39.32731
               exposure1 exposure2 exposure3
## F-statistic 126.7447 35.29937 39.32731
  • Fit a radial MVMR model
if (!requireNamespace("RMVMR", quietly = TRUE)) {
  remotes::install_github("WSpiller/RMVMR")
}
library(RMVMR)
rmvmr_input <- mrmvinput_to_rmvmr_format(datfmt)</pre>
rmvmr_fit <- ivw_rmvmr(rmvmr_input, summary = TRUE)</pre>
## Radial Multivariable MR
##
               Estimate Std. Error
                                    t value
                                                 Pr(>|t|)
##
## exposure1 0.4286200 0.0609661 7.030464 1.099077e-09
## exposure2 -0.1941989 0.1308289 -1.484372 1.421994e-01
## exposure3 0.2260456 0.1232828 1.833554 7.097168e-02
## Residual standard error: 1.49 on 70 degrees of freedom
rmvmr_fit$coef
##
               Estimate Std. Error
                                     t value
                                                 Pr(>|t|)
## exposure1 0.4286200 0.0609661 7.030464 1.099077e-09
## exposure2 -0.1941989 0.1308289 -1.484372 1.421994e-01
## exposure3 0.2260456 0.1232828 1.833554 7.097168e-02
  • Plot the radial MVMR models
plt_rmvmr <- plot_rmvmr(rmvmr_input, rmvmr = rmvmr_fit)</pre>
plt_rmvmr$p1
```

# Radial MVMR without correction



plt\_rmvmr\$p2





• Heterogeneity statistics

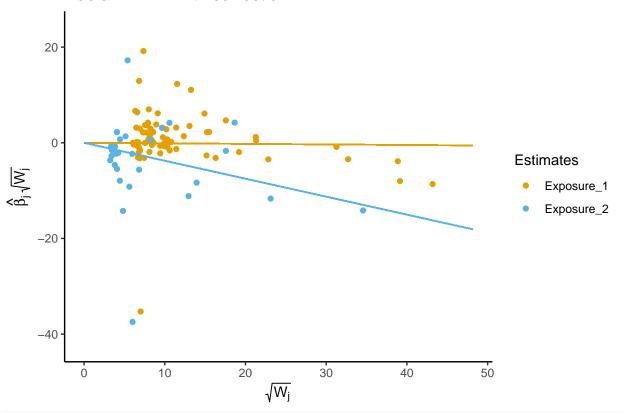
str\_rmvmr\$plot[[2]]

```
pleio_rmvmr <- pleiotropy_rmvmr(rmvmr_input, rmvmr = rmvmr_fit)</pre>
pleio_rmvmr$gq
##
               q_statistic
                                 p_value
## Exposure_1
                  76.37356 2.812609e-01
                  59.58894 8.243650e-06
## Exposure 2
## Exposure_3
                  45.88633 1.308596e-02
head(pleio_rmvmr$qdat)
##
                  wj corrected_beta
                                                        qj_p ref_exposure
                                              qj
## 1 snp_1 2.414215
                          0.3141338 0.031643343 0.85881269
                                                               Exposure_1
## 2 snp_2 3.938760
                          0.3821443 0.008507667 0.92650973
                                                               Exposure_1
## 3 snp_3 3.246657
                         -0.6057993 3.473998077 0.06234046
                                                               Exposure_1
                          0.3273484 0.020524059 0.88608310
## 4 snp_4 2.001191
                                                               Exposure_1
## 5 snp_5 9.278826
                          0.5414293 0.118081748 0.73112437
                                                               Exposure_1
                          1.0713541 0.544339092 0.46064001
## 6 snp_6 1.317671
                                                               Exposure_1
   • Conditional F-statistics for instrument strength (Sanderson, Spiller, and Bowden 2021)
str_rmvmr <- strength_rmvmr(rmvmr_input)</pre>
```

## each exposure not specified. Fixing covariance at 0.

## Warning in MVMR::strength\_mvmr(r\_input, gencov): Covariance between effect of genetic variants on

# Radial MVMR with correction



### str\_rmvmr\$qstat[[2]]

#### Stata code

• Load the Statamarkdown package to enable Stata code chunks in an R Markdown file

```
if (!requireNamespace("Statamarkdown", quietly = TRUE)) {
  remotes::install_github("Hemken/Statamarkdown")
}
library(Statamarkdown)
```

• Read in the data and create an indicator variable to select observations with p-value between the genotype and LDL-C < 10-8

```
use https://raw.github.com/remlapmot/mrrobust/master/dodata, clear
gen byte sel1 = (ldlcp2 < 1e-8)</pre>
```

### Example code using the mrrobust package

• Install the mrrobust package using the github package

```
// Note: output suppressed
net install mrrobust, from("https://raw.github.com/remlapmot/mrrobust/master/")
mrdeps
```

• Fit IVW (with fixed effect standard errors)

mregger chdbeta ldlcbeta [aw=1/(chdse^2)] if sel1==1, ivw fe

| Coef. Std. Err. z P>|z| [95% Conf. Interval] | Chdbeta | dlcbeta | .4815055 .038221 12.60 0.000 .4065938 .5564173

• Fit MR-Egger reporting I2GX statistic and heterogeneity Q-test

mregger chdbeta ldlcbeta [aw=1/(chdse^2)] if sel1==1, gxse(ldlcse) heterogi

Q\_GX statistic (weighted) = 3454.26

I^2\_GX statistic (weighted) = 97.92%

Number of genotypes = 73

Residual standard error = 1.548

Ruecker's Q for heterogeneity; chi2(71) = 170.11 (p = 0.0000)

I-squared statistic = 58.3% (95% CI 45.8%, 67.8%)

Coef. Std. Err. z P>|z| [95% Conf. Interval]

chdbeta | slope | .6173131 .1034573 5.97 0.000 .4145405 .8200858 \_cons | -.0087706 .0054812 -1.60 0.110 -.0195136 .0019723

• Simple plot of IVW and MR-Egger univariate estimates mreggerplot chdbeta chdse ldlcbeta ldlcse if sel1==1

• Adding the modal and median estimates onto the plot

| Coef. Std. Err. z P>|z| [95% Conf. Interval] | beta | .4582573 .0633137 7.24 0.000 .3341648 .5823499

Number of genotypes = 73
Replications = 1000

Phi = .25

 			[95% Conf.	Interval]
•			0232045	.8629471

• Fit and MVMR model with phenotypes LDL-c and HDL-c (Burgess, Dudbridge, and Thompson 2015).

mvmr chdbeta ldlcbeta hdlcbeta [aw=1/(chdse^2)] if sel1==1

Number of genotypes = 73 Number of phenotypes = 2 Standard errors: Random effect Residual standard error = 1.514

				[95% Conf.	Interval]
chdbeta   ldlcbeta	.0581901	8.03	0.000	.3530214 5305175	.5811224 0554921

• Additionally include a third phenotype – triglycerides.

mvmr chdbeta ldlcbeta hdlcbeta tgbeta [aw=1/(chdse^2)] if sel1==1

Number of genotypes = 73 Number of phenotypes = 3 Standard errors: Random effect Residual standard error = 1.490

	Coef.	Std. Err.				Interval]
chdbeta						
ldlcbeta	.42862	.0609661	7.03	0.000	.3091286	.5481113
hdlcbeta	1941989	.1308289	-1.48	0.138	4506189	.0622211
tgbeta	.2260456	.1232828	1.83	0.067	0155842	.4676755

• Report the QA statistic for instrument validity and the conditional F-statistics for instrument strength for each phenotype (Sanderson et al. 2019; Sanderson, Spiller, and Bowden 2021).

mvmr chdbeta ldlcbeta hdlcbeta tgbeta [aw=1/(chdse^2)] if sel1==1, gxse(ldlcse hdlcse tgse)

> e hdlcse tgse)

Number of genotypes = 73

Number of phenotypes = 3

Standard errors: Random effect
Residual standard error = 1.490

| Coef. Std. Err. z P>|z| [95% Conf. Interval]

chdbeta |

```
    ldlcbeta |
    .42862
    .0609661
    7.03
    0.000
    .3091286
    .5481113

    hdlcbeta |
    -.1941989
    .1308289
    -1.48
    0.138
    -.4506189
    .0622211

    tgbeta |
    .2260456
    .1232828
    1.83
    0.067
    -.0155842
    .4676755
```

\_\_\_\_\_\_

 $Q_A$  statistic for instrument validity; chi2(70) = 152.88 (p = 0.0000) Conditional F-statistics for instrument strength:

F x1 = 130.31 (ldlcbeta)

 $F_x2 = 36.29$  (hdlcbeta)

 $F_x3 = 40.44$  (tgbeta)

• Fit an MVMR-Egger regression (Rees, Wood, and Burgess 2017), orienting the model with respect to the first phenotype in the main *varlist*.

mrmvegger chdbeta ldlcbeta hdlcbeta tgbeta [aw=1/(chdse^2)] if sel1==1

MVMR-Egger model oriented wrt: ldlcbeta

Number of genotypes = 73

Number of phenotypes = 3

Residual standard error = 1.469

	Coef.	Std. Err.				Interval]
chdbeta						
ldlcbeta	.5672993	.1002611	5.66	0.000	.370791	.7638075
hdlcbeta	1364113	.1332727	-1.02	0.306	3976209	.1247983
tgbeta	.2739803	.1246927	2.20	0.028	.0295871	.5183735
_cons	0093655	.0054187	-1.73	0.084	019986	.001255

We can also orient the model with respect to HDL-C.

mrmvegger chdbeta ldlcbeta hdlcbeta tgbeta [aw=1/(chdse^2)] if sel1==1, orient(2)

> t(2)

MVMR-Egger model oriented wrt: hdlcbeta

Number of genotypes = 73

Number of phenotypes = 3

Residual standard error = 1.501

	Coef.	Std. Err.	z	P> z		Interval]
chdbeta						F40000
ldlcbeta   hdlcbeta		.0614056 .1541909	6.98 -1.29	0.000 0.197	.308287 5011723	.5489926 .1032449
tgbeta		.1243221	1.82	0.069 0.953	0179875 006883	.4693463
_cons	.0002155	.0030210	0.06	0.900	000003	.0073141

Or we can orient the model with respect to triglycerides.

mrmvegger chdbeta ldlcbeta hdlcbeta tgbeta [aw=1/(chdse^2)] if sel1==1, orient(3)

> t(3)

MVMR-Egger model oriented wrt: tgbeta Number of genotypes = 73 Number of phenotypes = 3

 	Coef.	Std. Err.	z	P> z		Interval]
chdbeta						
ldlcbeta	.4203073	.0660026	6.37	0.000	.2909447	.54967
hdlcbeta	1903089	.1321536	-1.44	0.150	4493252	.0687075
tgbeta	.2065651	.1365427	1.51	0.130	0610537	.474184
_cons	.0013499	.003951	0.34	0.733	0063939	.0090936

# R session information for reproducibility

\* 1.0.7

0.3.2

0.14

0.5.0

2.1.0

1.1.0

1.5.1

\* 0.5.1

```
library(sessioninfo)
session_info()
```

dplyr ellipsis

fansi

farver

fastmap

forcats

foreach

evaluate

```
- Session info ------
setting value
version R version 4.1.1 (2021-08-10)
         Windows 10 x64
system
         x86_64, mingw32
ui
         RTerm
language (EN)
 collate English_United Kingdom.1252
 ctype
         English_United Kingdom.1252
tz
         Europe/London
date
         2021-09-24
- Packages ------
package
                      * version date
                                          lib source
arrangements
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2020-10-15 [1] CRAN (R 4.1.0)

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                                  2020-10-31 [1] CRAN (R 4.1.0)
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```

```
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stringr
                      * 1.4.0
                               2019-02-10 [1] CRAN (R 4.1.0)
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tibble
                      * 3.1.4
                               2021-08-25 [1] CRAN (R 4.1.1)
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                     * 1.1.3 2021-03-03 [1] CRAN (R 4.1.0)
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                     * 1.3.1 2021-04-15 [1] CRAN (R 4.1.0)
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yaml
                        2.2.1 2020-02-01 [1] CRAN (R 4.1.0)
```

- [1] C:/Users/tom/Documents/R/win-library/4.1
- [2] C:/Program Files/R/R-4.1.1/library

# Stata session information for reproducibility

```
about
ado describe mrrobust
Stata/MP 16.1 for Windows (64-bit x86-64)
Revision 08 Jul 2021
Copyright 1985-2019 StataCorp LLC
Total physical memory:
                             32.00 GB
Available physical memory: 16.66 GB
Stata license: Unlimited-user 2-core network, expiring 21 Jan 2022
Serial number: 501609352178
  Licensed to: Tom Palmer
               University of Bristol
[40] package mrrobust from https://raw.githubusercontent.com/remlapmot/mrrobust
> /master
TITLE
      'mrrobust': Stata package for two-sample Mendelian randomization analyses
DESCRIPTION/AUTHOR(S)
      Author: Tom Palmer
      Distribution-Date: 20210917
INSTALLATION FILES
      m\mrmedian.ado
      m\mrmedian.sthlp
      m\mbox{\em mrmedianobs.ado}
      m\mrmedianobs_work.ado
```

```
m\mrmedianobs.sthlp
m\mregger.ado
m\mregger.sthlp
m\mrrobust.sthlp
m\mreggerplot.ado
m\mreggerplot.sthlp
m\mrmodal.ado
m\mrmodal.sthlp
m\mrratio.ado
m\mrratio.sthlp
m\mrivests.ado
m\mrivests.sthlp
m\mrforest.ado
m\mrforest.sthlp
m\mreggersimex.ado
m\mreggersimex.sthlp
m\mreggersimexonce.ado
m\mrmodalplot.ado
m\mrmodalplot.sthlp
m\mrfunnel.ado
m\mrfunnel.sthlp
m\mrdeps.ado
m\mrdeps.sthlp
m\mr.ado
m\mr.sthlp
m\mrmvivw.ado
m\mrmvivw.sthlp
m\mvivw.ado
m\mvivw.sthlp
m\mvmr.ado
m\mvmr.sthlp
m\mrmvegger.ado
m\mrmvegger.sthlp
m\mrleaveoneout.ado
m\mrleaveoneout.sthlp
m\mrrobust-author.ihlp
```

#### INSTALLED ON

24 Sep 2021

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#### References

Burgess, S, F Dudbridge, and SG Thompson. 2015. "Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects." *American Journal of Epidemiology* 181: 251--260. https://doi.org/10.1093/aje/kwu283.

Rees, J, A Wood, and S Burgess. 2017. "Extending the MR-Egger method for multivariable Mendelian randomization to correct for both measured and unmeasured pleiotropy." Statistics in Medicine 36: 4705–18. https://doi.org/10.1002/sim.7492.

Sanderson, E, G Davey Smith, F Windmeijer, and J Bowden. 2019. "An examination of multivariable Mendelian randomization in the single-sample and two-sample summary data settings." *International Journal of Epidemiology* 48: 713–27. https://doi.org/10.1093/ije/dyy262.

Sanderson, E, W Spiller, and J Bowden. 2021. "Testing and Correcting for Weak and Pleiotropic Instruments in Two-Sample Multivariable Mendelian Randomization." Statistics in Medicine. https://doi.org/10.1002/sim.9133.