Mendelian randomisation: Critical Covid-19 as exposure for stroke

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1. Preparation

Load the MendelianRandomization package for the Mendelian randomization (MR) analysis, metafor for the forestplots, cowplot for plotting and knitr and markdown for compilation.

Load the data which is provided in the Supplementary Table 2 available from this github repository.

```
data = read.csv("SupplementaryTable2.csv", stringsAsFactors=FALSE)
```

This dataset contains summary-level information on genetic associations with the exposure: - Critical Covid-19: beta_A2, se_A2, pval (from release 5 of https://www.covid19hg.org/results/r5/) and ischemic stroke outcomes including - Ischemic stroke (AIS): beta_ais, se_ais, p_ais - Cardioembolic stroke (CES): beta_ces, se_ces, p_ces - Large artery stroke (LAS): beta_las, se_las, p_las - Small vessel stroke (SVS): beta_svs, se_svs, p_svs

```
dim(data)
```

```
## [1] 31 21
```

```
head(data, n=5)
```

```
##   X        rsid CHR       POS REF ALT  beta_A2    se_A2     pval beta_ais
## 1 1  rs10087754   8 121819908   T   A -0.13356 0.026935 7.10e-07  -0.0119
## 2 2  rs11085727  19  10355447   C   T  0.17270 0.029295 3.74e-09  -0.0217
## 3 3 rs111508230   1 155181061   C   T -0.20553 0.043739 2.61e-06  -0.0081
## 4 4 rs114969787   5  65770656   C   T  0.30736 0.066335 3.60e-06   0.0409
## 5 5  rs11658357  17  36097317   A   T -0.20140 0.044070 4.88e-06   0.0097
##   se_ais   p_ais beta_ces se_ces   p_ces beta_las se_las   p_las beta_svs
## 1 0.0101 0.23740   0.0001 0.0194 0.99410  -0.0415 0.0250 0.09633  -0.0096
## 2 0.0112 0.05205  -0.0554 0.0218 0.01107  -0.0005 0.0275 0.98420   0.0349
## 3 0.0160 0.61430  -0.0708 0.0313 0.02366   0.0927 0.0390 0.01732   0.0285
## 4 0.0310 0.18670  -0.0468 0.0643 0.46620   0.1153 0.0773 0.13570   0.0437
## 5 0.0126 0.43780  -0.0184 0.0243 0.45020  -0.0154 0.0305 0.61260   0.0438
##   se_svs  p_svs
## 1 0.0233 0.6805
## 2 0.0254 0.1693
## 3 0.0405 0.4810
## 4 0.0724 0.5463
## 5 0.0288 0.1282
```

2. Main analysis

Defining the mr_input objects for each exposure.

```
mr_ais = mr_input(bx = data$beta_A2,bxse = data$se_A2, by = data$beta_ais, byse = data$se_ais)
mr_ces = mr_input(bx = data$beta_A2,bxse = data$se_A2, by = data$beta_ces, byse = data$se_ces)
mr_las = mr_input(bx = data$beta_A2,bxse = data$se_A2, by = data$beta_las, byse = data$se_las)
mr_svs = mr_input(bx = data$beta_A2,bxse = data$se_A2, by = data$beta_svs, byse = data$se_svs)
```

Compute the inverse-variance weighted (IVW) MR estimate.

```
ivw_ais = mr_ivw(mr_ais)
ivw_ces = mr_ivw(mr_ces)
ivw_las = mr_ivw(mr_las)
ivw_svs = mr_ivw(mr_svs)
```

IVW results including MR estimates, their standard error, confidence interval and heterogeneity statistics.

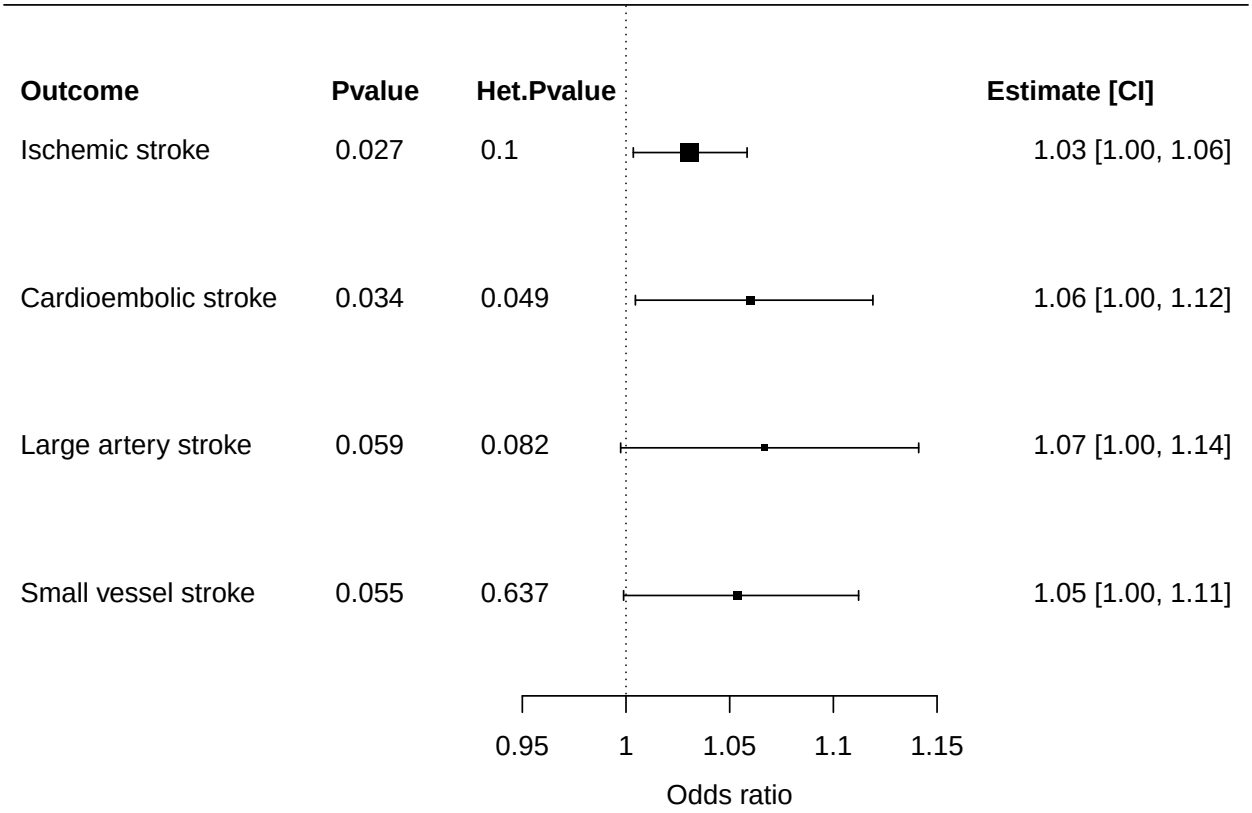
```
##                         Estimate   StdError      CILower    CIUpper     Pvalue
## Ischemic stroke      0.03012927 0.01358245  0.003508145 0.05675039 0.02653786
## Cardioembolic stroke 0.05850627 0.02754401  0.004521003 0.11249154 0.03366166
## Large artery stroke  0.06473085 0.03434099 -0.002576245 0.13203795 0.05943734
## Small vessel stroke  0.05257574 0.02741739 -0.001161359 0.10631284 0.05516105
##                         Q-stat Heter.Pvalue
## Ischemic stroke      40.23033   0.10047977
## Cardioembolic stroke 43.89899   0.04870776
## Large artery stroke  41.28956   0.08220629
## Small vessel stroke  26.74162   0.63680388
```

In the manuscript we present the MR estimates and confidence intervalls on the odds ratio scale, where MR estimates represent the odds ratio for critical Covid-19 per unit increase in the log odds ratio of stroke phenotypes.

```
##                      Estimate   StdError   CILower  CIUpper     Pvalue   Q-stat
## Ischemic stroke      1.030588 0.01358245 1.0035143 1.058392 0.02653786 40.23033
## Cardioembolic stroke 1.060252 0.02754401 1.0045312 1.119063 0.03366166 43.89899
## Large artery stroke  1.066872 0.03434099 0.9974271 1.141152 0.05943734 41.28956
## Small vessel stroke  1.053982 0.02741739 0.9988393 1.112170 0.05516105 26.74162
##                      Heter.Pvalue
## Ischemic stroke        0.10047977
## Cardioembolic stroke   0.04870776
## Large artery stroke    0.08220629
## Small vessel stroke    0.63680388
```

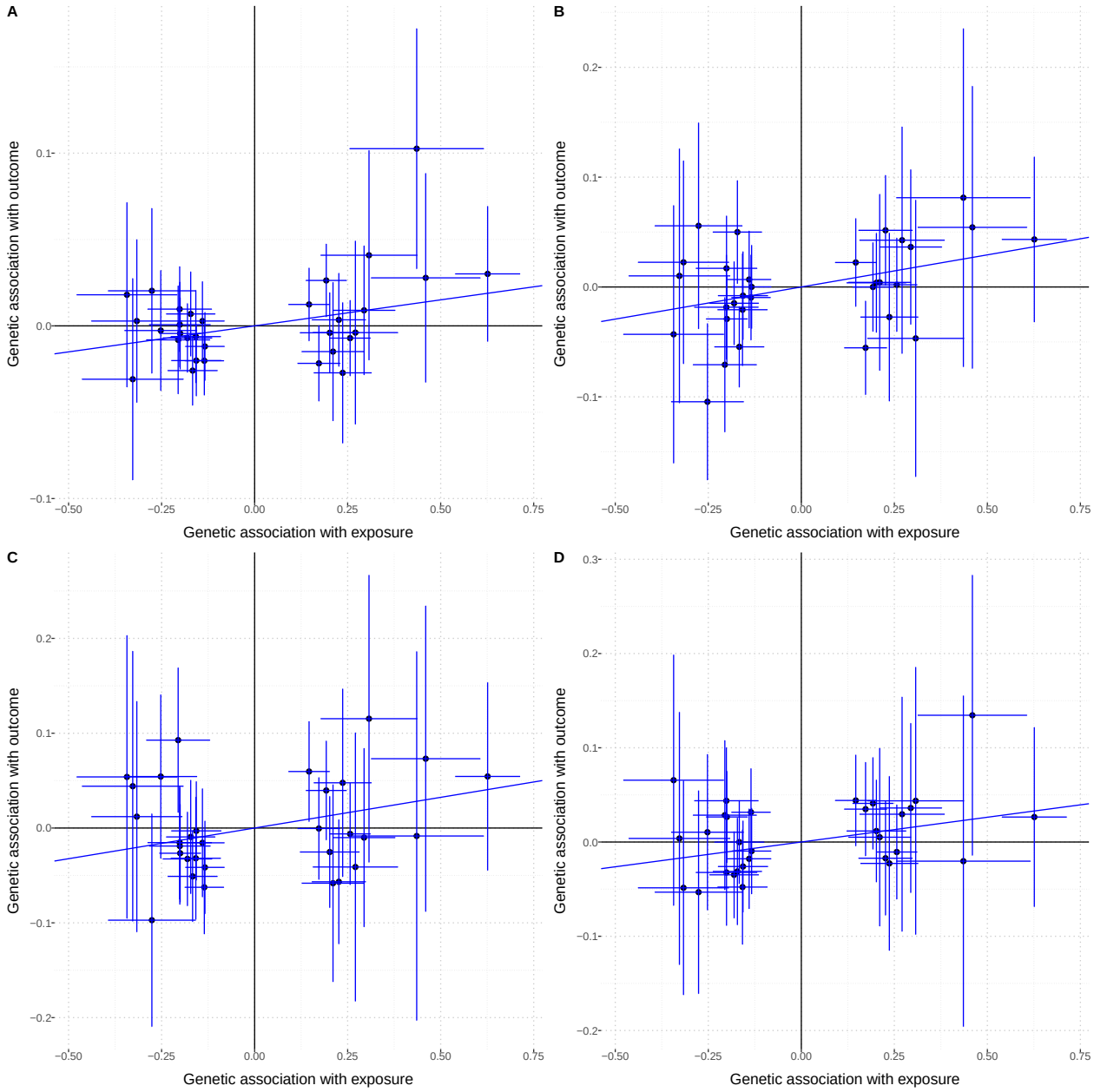
Figure 2: Forest plot of MR estimates.

```
tableIVW = as.data.frame(tableIVW)
tableIVW$Pvalue = round(tableIVW$Pvalue, digits=3)
tableIVW$Heter.Pvalue = round(tableIVW$Heter.Pvalue, digits=3)
forest(x=tableIVW$Estimate,ci.lb=tableIVW$CILower, ci.ub=tableIVW$CIUpper,
    refline=1,xlab="Odds ratio",slab=rownames(tableIVW),transf=exp, digits=2L, top=1,
    ilab=cbind(tableIVW$Pvalue, tableIVW$Heter.Pvalue), ilab.xpos=c(0.852,0.922),
    ilab.pos=4, xlim=c(0.7,1.3))
text(c(0.7,0.85,0.92,1.166), 4.4, pos=4,
 c("Outcome","Pvalue","Het.Pvalue", "Estimate [CI]"),font=2)
```



Supplementary Figure 1: Scatterplots of genetic association with the exposure (critical Covid-19) against the genetic association with the outcome (ischemic stroke).

```
m1 = mr_plot(mr_ais, interactive = FALSE)
m2 = mr_plot(mr_ces, interactive = FALSE)
m3 = mr_plot(mr_las, interactive = FALSE)
m4 = mr_plot(mr_svs, interactive = FALSE)
plot_grid(m1,m2,m3,m4, labels = c('A', 'B', 'C', 'D'),nrow = 2, ncol = 2)
```



3. Sensitivity (Pleiotropy robust approaches)

Compute as sensitivity pleiotropy-robust MR estimates.

```
sensitivity_ais = mr_allmethods(mr_ais, method = "main")
sensitivity_ces = mr_allmethods(mr_ces, method = "main")
sensitivity_las = mr_allmethods(mr_las, method = "main")
sensitivity_svs = mr_allmethods(mr_svs, method = "main")
sensitivity = rbind(sensitivity_ais$Values,sensitivity_ces$Values,
 sensitivity_las$Values, sensitivity_svs$Values)
colnames(sensitivity)[4:5] = c("CILower","CIUpper")
sensitivity
```

```
##             Method     Estimate   Std Error       CILower    CIUpper    P-value
## 1    Simple median  0.021987907 0.018206992 -0.0136971415 0.05767295 0.22717699
## 2  Weighted median  0.030696306 0.018020121 -0.0046224833 0.06601509 0.08848462
## 3              IVW  0.030129267 0.013582455  0.0035081452 0.05675039 0.02653786
## 4         MR-Egger  0.021878677 0.038831730 -0.0542301156 0.09798747 0.57314711
## 5      (intercept)  0.001878140 0.008262309 -0.0143156891 0.01807197 0.82017946
## 6    Simple median  0.069150550 0.034830146  0.0008847178 0.13741638 0.04710389
## 7  Weighted median  0.069257467 0.034694043  0.0012583917 0.13725654 0.04590834
## 8              IVW  0.058506270 0.027544010  0.0045210033 0.11249154 0.03366166
## 9         MR-Egger  0.088183516 0.079298775 -0.0672392275 0.24360626 0.26612038
## 10     (intercept) -0.006694408 0.016740805 -0.0395057836 0.02611697 0.68924072
## 11   Simple median  0.076464747 0.045306995 -0.0123353325 0.16526483 0.09146828
## 12 Weighted median  0.083237696 0.045351215 -0.0056490524 0.17212444 0.06644620
## 13             IVW  0.064730851 0.034340986 -0.0025762447 0.13203795 0.05943734
## 14        MR-Egger -0.084847837 0.095521477 -0.2720664915 0.10237082 0.37440130
## 15     (intercept)  0.033683998 0.020156359 -0.0058217403 0.07318974 0.09469501
## 16   Simple median  0.071877808 0.041936068 -0.0103153746 0.15407099 0.08653141
## 17 Weighted median  0.049359765 0.041708370 -0.0323871371 0.13110667 0.23663090
## 18             IVW  0.052575743 0.027417392 -0.0011613586 0.10631284 0.05516105
## 19        MR-Egger  0.011996426 0.079115104 -0.1430663284 0.16705918 0.87947675
## 20     (intercept)  0.009100370 0.016642977 -0.0235192663 0.04172001 0.58451657
```

None of the MR-Egger estimates is significantly difference from zero.

```
MREgger_intercept = sensitivity[c(5,10,15,20),]
rownames(MREgger_intercept) = c("Ischemic stroke","Cardioembolic stroke","Large artery stroke","Small ve
MREgger_intercept
```

```
##                          Method     Estimate   Std Error     CILower
## Ischemic stroke      (intercept)  0.001878140 0.008262309 -0.01431569
## Cardioembolic stroke (intercept) -0.006694408 0.016740805 -0.03950578
## Large artery stroke  (intercept)  0.033683998 0.020156359 -0.00582174
## Small vessel stroke  (intercept)  0.009100370 0.016642977 -0.02351927
##                         CIUpper    P-value
## Ischemic stroke      0.01807197 0.82017946
## Cardioembolic stroke 0.02611697 0.68924072
## Large artery stroke  0.07318974 0.09469501
## Small vessel stroke  0.04172001 0.58451657
```

Transform estimates to the odds-ratio scale.

```
##             Method  Estimate   CILower  CIUpper    P.value
## 1    Simple median 1.0222314 0.9863962 1.059368 0.22717699
## 2  Weighted median 1.0311723 0.9953882 1.068243 0.08848462
## 3              IVW 1.0305877 1.0035143 1.058392 0.02653786
## 4         MR-Egger 1.0221198 0.9472141 1.102949 0.57314711
## 6    Simple median 1.0715975 1.0008851 1.147306 0.04710389
## 7  Weighted median 1.0717121 1.0012592 1.147122 0.04590834
## 8              IVW 1.0602516 1.0045312 1.119063 0.03366166
## 9         MR-Egger 1.0921885 0.9349715 1.275842 0.26612038
## 11   Simple median 1.0794641 0.9877404 1.179705 0.09146828
## 12 Weighted median 1.0868001 0.9943669 1.187826 0.06644620
## 13             IVW 1.0668718 0.9974271 1.141152 0.05943734
## 14        MR-Egger 0.9186521 0.7618036 1.107794 0.37440130
## 16   Simple median 1.0745240 0.9897376 1.166574 0.08653141
## 17 Weighted median 1.0505983 0.9681317 1.140089 0.23663090
```

```
## 18             IVW 1.0539824 0.9988393 1.112170 0.05516105
## 19        MR-Egger 1.0120687 0.8666966 1.181824 0.87947675
```

4. Sensitivity (Genome-wide significant IVs)

Defining the mr_input objects for each exposure on genome-wide significant genetic variants as instrumental variables only.

```
data = data[data$pval<5e-8,]
dim(data)
```

```
## [1]  9 21
```

```
mr2_ais = mr_input(bx = data$beta_A2,bxse = data$se_A2, by = data$beta_ais, byse = data$se_ais)
mr2_ces = mr_input(bx = data$beta_A2,bxse = data$se_A2, by = data$beta_ces, byse = data$se_ces)
mr2_las = mr_input(bx = data$beta_A2,bxse = data$se_A2, by = data$beta_las, byse = data$se_las)
mr2_svs = mr_input(bx = data$beta_A2,bxse = data$se_A2, by = data$beta_svs, byse = data$se_svs)
ivw2_ais = mr_ivw(mr2_ais)
ivw2_ces = mr_ivw(mr2_ces)
ivw2_las = mr_ivw(mr2_las)
ivw2_svs = mr_ivw(mr2_svs)
```

IVW results including MR estimates, their standard error, confidence interval and heterogeneity statistics.

```
##                        Estimate   StdError     CILower    CIUpper    Pvalue
## Ischemic stroke      0.01951162 0.02318834 -0.02593668 0.06495993 0.4001008
## Cardioembolic stroke 0.04663039 0.04477445 -0.04112592 0.13438671 0.2976663
## Large artery stroke  0.04954678 0.04360964 -0.03592654 0.13502011 0.2558968
## Small vessel stroke  0.04305075 0.04451297 -0.04419307 0.13029456 0.3334687
##                         Q-stat Heter.Pvalue
## Ischemic stroke      14.082702   0.07963513
## Cardioembolic stroke 14.137542   0.07825014
## Large artery stroke   7.717838   0.46150661
## Small vessel stroke   9.417210   0.30833142
```

In the manuscript we present the MR estimates and confidence intervalls on the odds ratio scale, where MR estimates represent the odds ratio for critical Covid-19 per unit increase in the log odds ratio of stroke phenotypes.

```
##                       Estimate   StdError   CILower  CIUpper    Pvalue    Q-stat
## Ischemic stroke      1.019703 0.02318834 0.9743968 1.067116 0.4001008 14.082702
## Cardioembolic stroke 1.047735 0.04477445 0.9597083 1.143835 0.2976663 14.137542
## Large artery stroke  1.050795 0.04360964 0.9647112 1.144560 0.2558968  7.717838
## Small vessel stroke  1.043991 0.04451297 0.9567692 1.139164 0.3334687  9.417210
##                       Heter.Pvalue
## Ischemic stroke         0.07963513
## Cardioembolic stroke    0.07825014
## Large artery stroke     0.46150661
## Small vessel stroke     0.30833142
```

Supplementary Figure 3: Forest plot of MR estimates (genome-wide significant genetic variants as instrumental variables).

```
table2IVW = as.data.frame(table2IVW)
table2IVW$Pvalue = round(table2IVW$Pvalue, digits=3)
table2IVW$Heter.Pvalue = round(table2IVW$Heter.Pvalue, digits=3)
```

```
forest(x=table2IVW$Estimate,ci.lb=table2IVW$CILower, ci.ub=table2IVW$CIUpper,
    refline=1,xlab="Odds ratio",slab=rownames(table2IVW),transf=exp, digits=3L, top=1,
    ilab=cbind(table2IVW$Pvalue, table2IVW$Heter.Pvalue), ilab.xpos=c(0.835,0.89),
    ilab.pos=4, xlim=c(0.7,1.28))
text(c(0.7,0.831,0.886,1.148), 4.4, pos=4,
 c("Outcome","Pvalue","Het.Pvalue", "Estimate [CI]"),font=2)
```