

Neutrophil count and breast cancer

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```
library(MendelianRandomization)
library(TwoSampleMR)
```

```
## TwoSampleMR version 0.5.4
## [>] All datasets re-instated
## [>] New: Option to use non-European LD reference panels for clumping etc
## [>] See news(package='TwoSampleMR') and https://gwas.mrcieu.ac.uk for latest information

##
## Attaching package: 'TwoSampleMR'

## The following objects are masked from 'package:MendelianRandomization':
##
##      mr_ivw, mr_median
```

```
library(MRInstruments)
```

data catalogue GWAS summary stats

```
data("gwas_catalog")
```

Exposure selection

```
###exposure_gwas <- subset(gwas_catalog, grepl("Astle", Author))

exposure_gwas <- subset(gwas_catalog, grepl("Astle", Author) &
  Phenotype_simple == "Neutrophil count")
```

```
head(exposure_gwas[,c(7:12,18:21)])
```

```
##           Phenotype Phenotype_info PubmedID   Author Year      SNP
## 772   Neutrophil count                27863252 Astle WJ 2016 rs10075801
## 1417  Neutrophil count                27863252 Astle WJ 2016 rs10138752
## 1747  Neutrophil count                27863252 Astle WJ 2016 rs10171849
## 1770  Neutrophil count                27863252 Astle WJ 2016 rs10173538
```

```
## 2746 Neutrophil count          27863252 Astle WJ 2016 rs1025688
## 3353 Neutrophil count          27863252 Astle WJ 2016 rs10409243
##      effect_allele other_allele      beta      se
## 772      G      A -0.02915474 0.003571429
## 1417     T      C -0.04593487 0.006632653
## 1747     C      A  0.03451534 0.003826531
## 1770     T    <NA>  0.02615576 0.003571429
## 2746     A      G  0.02332930 0.003826531
## 3353     T    <NA> -0.02207318 0.003571429
```

Excluding exposure values $>5 \times 10^8$

```
exposure_gwas<-exposure_gwas[exposure_gwas$pval<5*10^-8,]
```

```
head(exposure_gwas[,c(7:12,18:21)])
```

```
##      Phenotype Phenotype_info PubmedID  Author Year      SNP
## 772 Neutrophil count          27863252 Astle WJ 2016 rs10075801
## 1417 Neutrophil count          27863252 Astle WJ 2016 rs10138752
## 1747 Neutrophil count          27863252 Astle WJ 2016 rs10171849
## 1770 Neutrophil count          27863252 Astle WJ 2016 rs10173538
## 2746 Neutrophil count          27863252 Astle WJ 2016 rs1025688
## 3353 Neutrophil count          27863252 Astle WJ 2016 rs10409243
##      effect_allele other_allele      beta      se
## 772      G      A -0.02915474 0.003571429
## 1417     T      C -0.04593487 0.006632653
## 1747     C      A  0.03451534 0.003826531
## 1770     T    <NA>  0.02615576 0.003571429
## 2746     A      G  0.02332930 0.003826531
## 3353     T    <NA> -0.02207318 0.003571429
```

```
exposure_data<-format_data(exposure_gwas)
```

```
## Warning in format_data(exposure_gwas): other_allele column has some values that
## are not A/C/T/G or an indel comprising only these characters or D/I. These SNPs
## will be excluded
```

```
## Warning in .fun(piece, ...): More than one type of unit specified for Neutrophil
## count
```

```
## Warning in format_data(exposure_gwas): The following SNP(s) are missing required information for the
## rs10173538
## rs10409243
## rs11104881
## rs11359909
## rs11428934
## rs1144700
## rs11734460
## rs11741826
## rs12600856
## rs12905525
```

```

## rs139707092
## rs140578741
## rs14408
## rs145013566
## rs146318841
## rs147694761
## rs192022
## rs200638392
## rs201399927
## rs2208568
## rs2246941
## rs2979489
## rs3184504
## rs34061361
## rs34115320
## rs34208856
## rs34762051
## rs35188965
## rs35955747
## rs3917914
## rs397731840
## rs397933924
## rs398032702
## rs4147915
## rs552557467
## rs575404063
## rs58548501
## rs635634
## rs6500550
## rs71191701
## rs74267027
## rs7705526
## rs791357
## rs796415138
## rs8068017
## rs8179
## rs8705

```

```
#####Clumping data
```

```
exposure_data<-clump_data(exposure_data, clump_r2 = 0.001)
```

```
## API: public: http://gwas-api.mrcieu.ac.uk/
```

```
## Clumping nIt4Xv, 74 variants, using EUR population reference
```

```
## Removing 23 of 74 variants due to LD with other variants or absence from LD reference panel
```

```
## Clumping d8Rvo4, 73 variants, using EUR population reference
```

```
## Removing 20 of 73 variants due to LD with other variants or absence from LD reference panel
```

Outcome selection

```
ao<-available_outcomes()
head(ao)
```

```
## # A tibble: 6 x 19
##   id    trait sample_size   nsnp   year consortium author note    mr sex
##   <chr> <chr>      <int>  <int> <int> <chr>      <chr> <chr> <int> <chr>
## 1 eqtl~ ENSG~      4677 1.76e4 2018 NA          Vosa U NA      1 Male~
## 2 eqtl~ ENSG~      4994 1.78e4 2018 NA          Vosa U NA      1 Male~
## 3 eqtl~ ENSG~      4169 1.61e4 2018 NA          Vosa U NA      1 Male~
## 4 ukb~ 3mm ~      94258 9.85e6 2018 MRC-IEU Ben E~ 5159~    1 Male~
## 5 eqtl~ ENSG~      31684 1.74e4 2018 NA          Vosa U NA      1 Male~
## 6 ukb~ Body~      336107 1.09e7 2017 Neale Lab Neale NA      1 Male~
## # ... with 9 more variables: priority <int>, population <chr>, unit <chr>,
## #   subcategory <chr>, category <chr>, pmid <int>, ncase <int>, ncontrol <int>,
## #   sd <dbl>
```

###Select trait

```
outcome_gwas <- subset(ao, grepl("Breast cancer", trait))
head(outcome_gwas)
```

```
## # A tibble: 6 x 19
##   id    trait sample_size   nsnp   year consortium author note    mr sex
##   <chr> <chr>      <int>  <int> <int> <chr>      <chr> <chr> <int> <chr>
## 1 ieu~ Brea~      89677 1.96e5 2015 NA          Micha~ <NA>    1 Fema~
## 2 ieu~ ER+ ~      175475 1.07e7 2017 NA          Micha~ <NA>    1 Fema~
## 3 ieu~ Brea~      106776 1.07e7 2017 NA          Micha~ <NA>    1 Fema~
## 4 ukb~ Illn~      423458 9.85e6 2018 MRC-IEU Ben E~ 2011~    1 Male~
## 5 ieu~ ER+ ~      69970 1.07e7 2017 NA          Micha~ <NA>    1 Fema~
## 6 ieu~ Brea~      228951 1.07e7 2017 NA          Micha~ <NA>    1 Fema~
## # ... with 9 more variables: priority <int>, population <chr>, unit <chr>,
## #   subcategory <chr>, category <chr>, pmid <int>, ncase <int>, ncontrol <int>,
## #   sd <dbl>
```

##Selecting the exposure data

```
outcome_data <- extract_outcome_data(
  snps = exposure_data$SNP, outcomes = "ieu-a-1126")
```

Extracting data for 104 SNP(s) from 1 GWAS(s)

Finding proxies for 5 SNPs in outcome ieu-a-1126

Extracting data for 5 SNP(s) from 1 GWAS(s)

###Harmonize data

```

H_data <- harmonise_data(
  exposure_dat = exposure_data,
  outcome_dat = outcome_data
)

## Harmonising Neutrophil count (unit decrease) (nIt4Xv) and Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis)

## Removing the following SNPs for incompatible alleles:
## rs11741826

## Removing the following SNPs for being palindromic with intermediate allele frequencies:
## rs11741826, rs35188965, rs3917932

## Harmonising Neutrophil count (unit increase) (d8Rvo4) and Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis)

## Removing the following SNPs for incompatible alleles:
## rs192022, rs35955747, rs71191701

## Removing the following SNPs for being palindromic with intermediate allele frequencies:
## rs192022, rs35955747, rs71191701

####MR analysis

mr_results<-mr(H_data)

## Analysing 'd8Rvo4' on 'ieu-a-1126'

## Analysing 'nIt4Xv' on 'ieu-a-1126'

mr_results

##      id.exposure id.outcome
## 1      d8Rvo4 ieu-a-1126
## 2      d8Rvo4 ieu-a-1126
## 3      d8Rvo4 ieu-a-1126
## 4      d8Rvo4 ieu-a-1126
## 5      d8Rvo4 ieu-a-1126
## 6      nIt4Xv ieu-a-1126
## 7      nIt4Xv ieu-a-1126
## 8      nIt4Xv ieu-a-1126
## 9      nIt4Xv ieu-a-1126
## 10     nIt4Xv ieu-a-1126
##
##                                     outcome
## 1 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 2 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 3 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 4 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 5 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 6 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 7 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126

```

```
## 8 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 9 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 10 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
##           exposure                      method nsnp          b
## 1 Neutrophil count (unit increase)          MR Egger    49 -0.02062900
## 2 Neutrophil count (unit increase)      Weighted median    49 -0.06640771
## 3 Neutrophil count (unit increase) Inverse variance weighted    49 -0.03661084
## 4 Neutrophil count (unit increase)          Simple mode    49 -0.03405338
## 5 Neutrophil count (unit increase)      Weighted mode    49 -0.05174669
## 6 Neutrophil count (unit decrease)          MR Egger    45  0.08637773
## 7 Neutrophil count (unit decrease)      Weighted median    45  0.03281459
## 8 Neutrophil count (unit decrease) Inverse variance weighted    45  0.01554233
## 9 Neutrophil count (unit decrease)          Simple mode    45  0.01252482
## 10 Neutrophil count (unit decrease)      Weighted mode    45  0.02745049
##           se      pval
## 1 0.09182045 0.8232125
## 2 0.04074430 0.1031300
## 3 0.04348850 0.3998713
## 4 0.07504594 0.6520415
## 5 0.03675110 0.1655659
## 6 0.15606749 0.5828123
## 7 0.04974326 0.5094599
## 8 0.06419176 0.8086845
## 9 0.09104892 0.8912147
## 10 0.06741547 0.6858471
```

###CI generation

```
generate_odds_ratios(mr_results)
```

```
##      id.exposure id.outcome
## 1      d8Rvo4 ieu-a-1126
## 2      d8Rvo4 ieu-a-1126
## 3      d8Rvo4 ieu-a-1126
## 4      d8Rvo4 ieu-a-1126
## 5      d8Rvo4 ieu-a-1126
## 6      nIt4Xv ieu-a-1126
## 7      nIt4Xv ieu-a-1126
## 8      nIt4Xv ieu-a-1126
## 9      nIt4Xv ieu-a-1126
## 10     nIt4Xv ieu-a-1126
##                                     outcome
## 1 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 2 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 3 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 4 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 5 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 6 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 7 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 8 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 9 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 10 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
##           exposure                      method nsnp          b
```

```
## 1 Neutrophil count (unit increase) MR Egger 49 -0.02062900
## 2 Neutrophil count (unit increase) Weighted median 49 -0.06640771
## 3 Neutrophil count (unit increase) Inverse variance weighted 49 -0.03661084
## 4 Neutrophil count (unit increase) Simple mode 49 -0.03405338
## 5 Neutrophil count (unit increase) Weighted mode 49 -0.05174669
## 6 Neutrophil count (unit decrease) MR Egger 45 0.08637773
## 7 Neutrophil count (unit decrease) Weighted median 45 0.03281459
## 8 Neutrophil count (unit decrease) Inverse variance weighted 45 0.01554233
## 9 Neutrophil count (unit decrease) Simple mode 45 0.01252482
## 10 Neutrophil count (unit decrease) Weighted mode 45 0.02745049
##      se      pval      lo_ci      up_ci      or      or_lci95      or_uci95
## 1 0.09182045 0.8232125 -0.20059708 0.15933909 0.9795823 0.8182420 1.172736
## 2 0.04074430 0.1031300 -0.14626654 0.01345111 0.9357493 0.8639274 1.013542
## 3 0.04348850 0.3998713 -0.12184830 0.04862661 0.9640512 0.8852827 1.049828
## 4 0.07504594 0.6520415 -0.18114342 0.11303666 0.9665199 0.8343157 1.119673
## 5 0.03675110 0.1655659 -0.12377885 0.02028547 0.9495694 0.8835752 1.020493
## 6 0.15606749 0.5828123 -0.21951455 0.39227001 1.0902181 0.8029085 1.480337
## 7 0.04974326 0.5094599 -0.06468221 0.13031138 1.0333589 0.9373653 1.139183
## 8 0.06419176 0.8086845 -0.11027351 0.14135818 1.0156637 0.8955891 1.151837
## 9 0.09104892 0.8912147 -0.16593107 0.19098070 1.0126036 0.8471046 1.210436
## 10 0.06741547 0.6858471 -0.10468382 0.15958481 1.0278307 0.9006092 1.173024
```

##Sensitivity

```
mr_pleiotropy_test(H_data)
```

```
##      id.exposure id.outcome
## 1      d8Rvo4 ieu-a-1126
## 2      nIt4Xv ieu-a-1126
##
##                                     outcome
## 1 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 2 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
##
##      exposure egger_intercept      se      pval
## 1 Neutrophil count (unit increase) -0.0007820908 0.003945702 0.8437330
## 2 Neutrophil count (unit decrease) -0.0029567290 0.005927334 0.6204419
```

###Heterogeneity

```
mr_heterogeneity(H_data, method_list=c("mr_egger_regression", "mr_ivw"))
```

```
##      id.exposure id.outcome
## 1      d8Rvo4 ieu-a-1126
## 2      d8Rvo4 ieu-a-1126
## 3      nIt4Xv ieu-a-1126
## 4      nIt4Xv ieu-a-1126
##
##                                     outcome
## 1 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 2 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 3 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
## 4 Breast cancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ieu-a-1126
##
##      exposure      method      Q      Q_df
## 1 Neutrophil count (unit increase) MR Egger 152.4742 47
```

```
## 2 Neutrophil count (unit increase) Inverse variance weighted 152.6017 48
## 3 Neutrophil count (unit decrease) MR Egger 191.5185 43
## 4 Neutrophil count (unit decrease) Inverse variance weighted 192.6268 44
## Q_pval
## 1 4.558309e-13
## 2 7.963672e-13
## 3 1.216015e-20
## 4 1.682518e-20
```

```
###Scatterplot
```

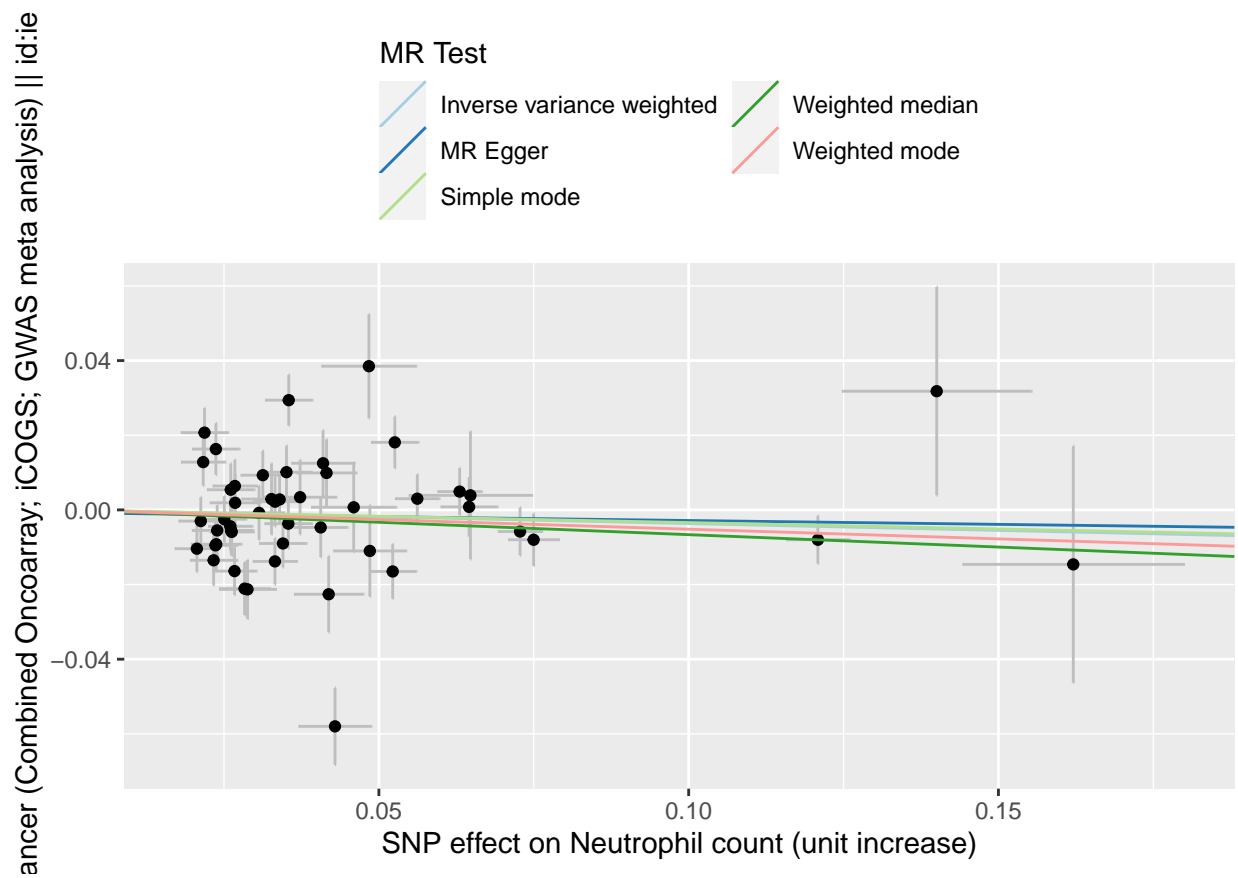
```
plot1 <- mr_scatter_plot(mr_results, H_data)
```

```
## Warning: Ignoring unknown aesthetics: text
```

```
## Warning: Ignoring unknown aesthetics: text
```

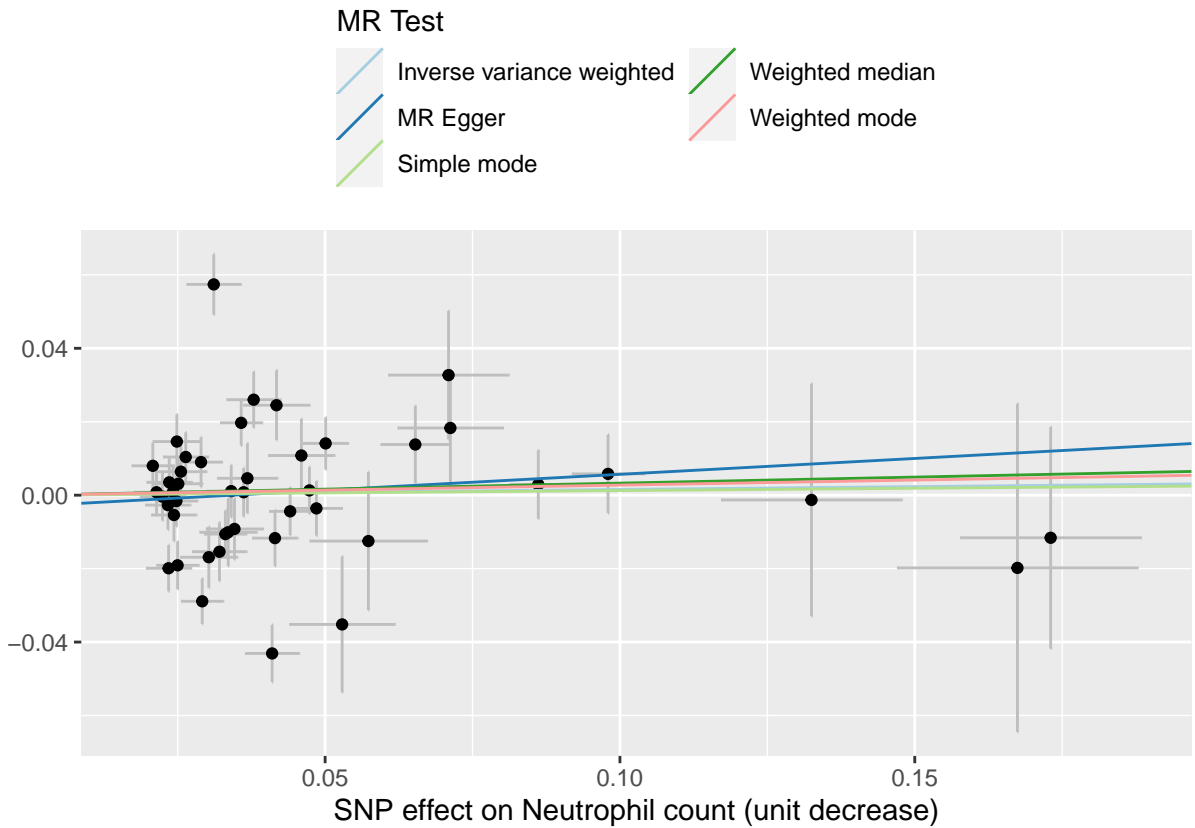
```
plot1
```

```
## $'d8Rvo4.ieu-a-1126'
```



```
## $'nIt4Xv.ieu-a-1126'
```


ancer (Combined Oncoarray; iCOGS; GWAS meta analysis) || id:ie



```
##
## attr("split_type")
## [1] "data.frame"
## attr("split_labels")
##   id.exposure id.outcome
## 1      d8Rvo4 ieu-a-1126
## 2      nIt4Xv ieu-a-1126
```

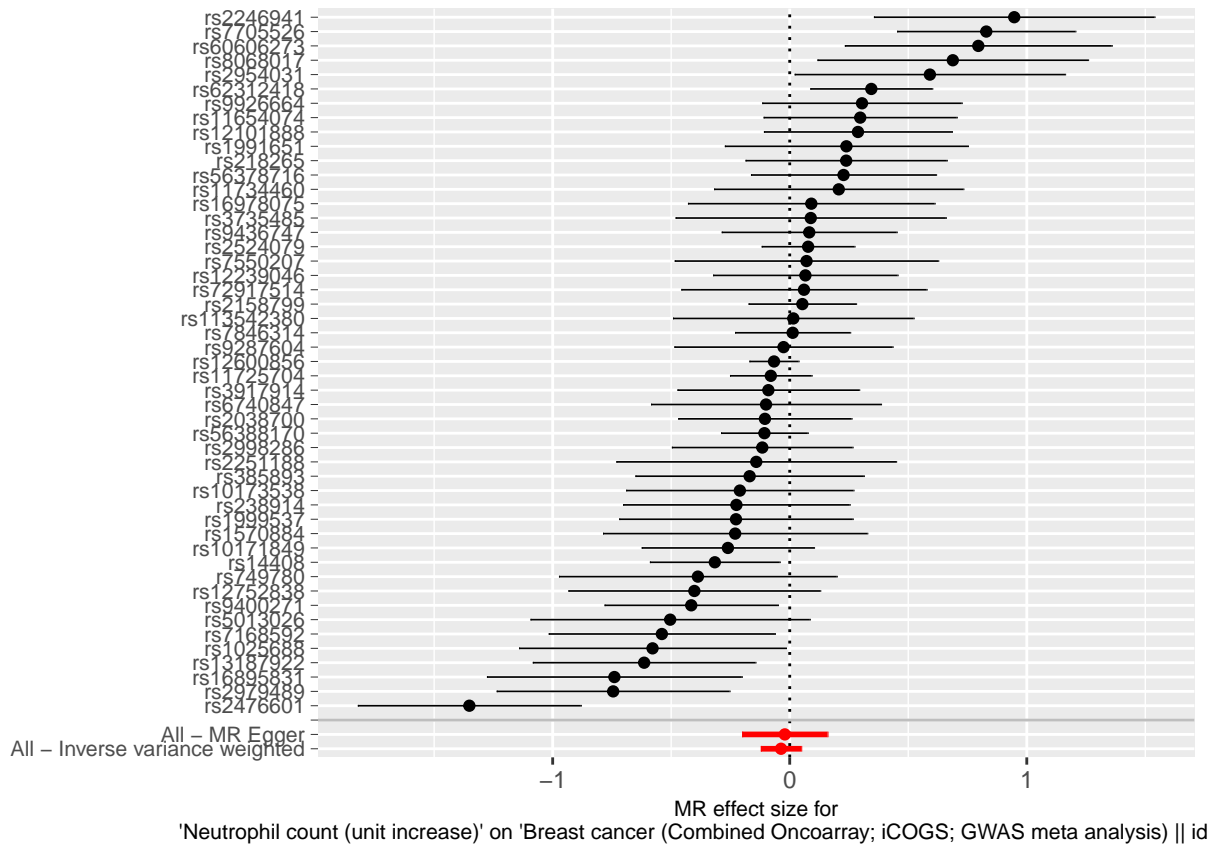
Forest Plot individual SNPs

```
res_single <- mr_singlesnp(H_data)
plot2 <- mr_forest_plot(res_single)
plot2
```

```
## $'d8Rvo4.ieu-a-1126'
```

```
## Warning: Removed 1 rows containing missing values (geom_errorbarh).
```

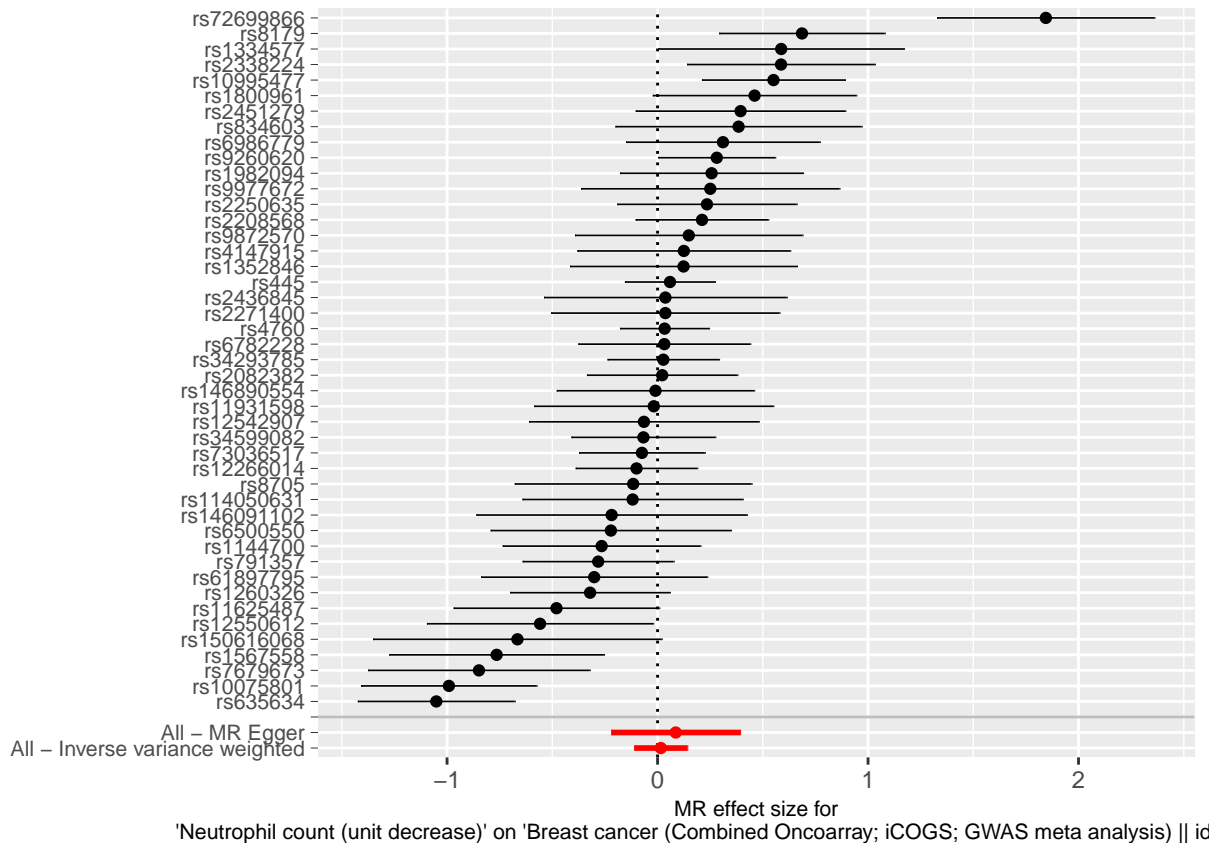
```
## Warning: Removed 1 rows containing missing values (geom_point).
```



```
##
## $'nIt4Xv.ieu-a-1126'

## Warning: Removed 1 rows containing missing values (geom_errorbarh).

## Warning: Removed 1 rows containing missing values (geom_point).
```



```
##
## attr("split_type")
## [1] "data.frame"
## attr("split_labels")
##   id.exposure id.outcome
## 1      d8Rvo4 ieu-a-1126
## 2      nIt4Xv ieu-a-1126
```

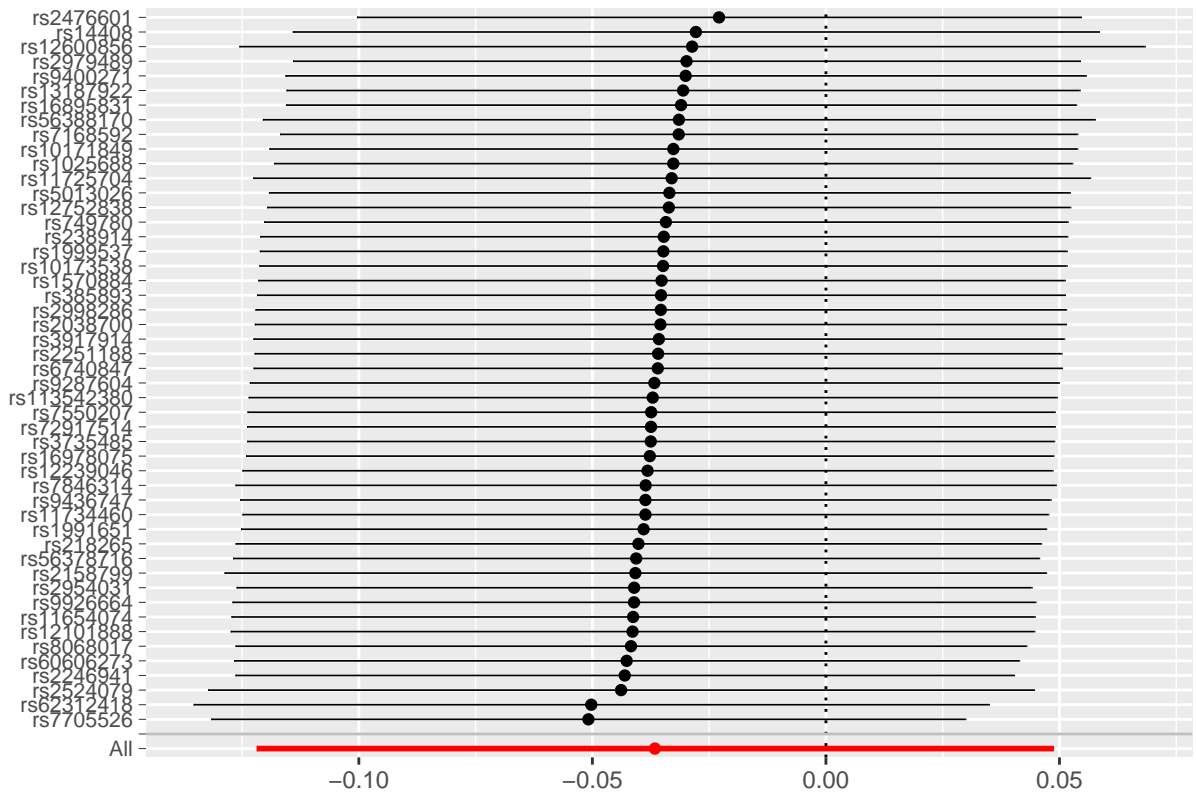
```
###Leave one out analysis
```

```
res_loo <- mr_leaveoneout(H_data)
plot3 <- mr_leaveoneout_plot(res_loo)
plot3
```

```
## $'d8Rvo4.ieu-a-1126'
```

```
## Warning: Removed 1 rows containing missing values (geom_errorbarh).
```

```
## Warning: Removed 1 rows containing missing values (geom_point).
```

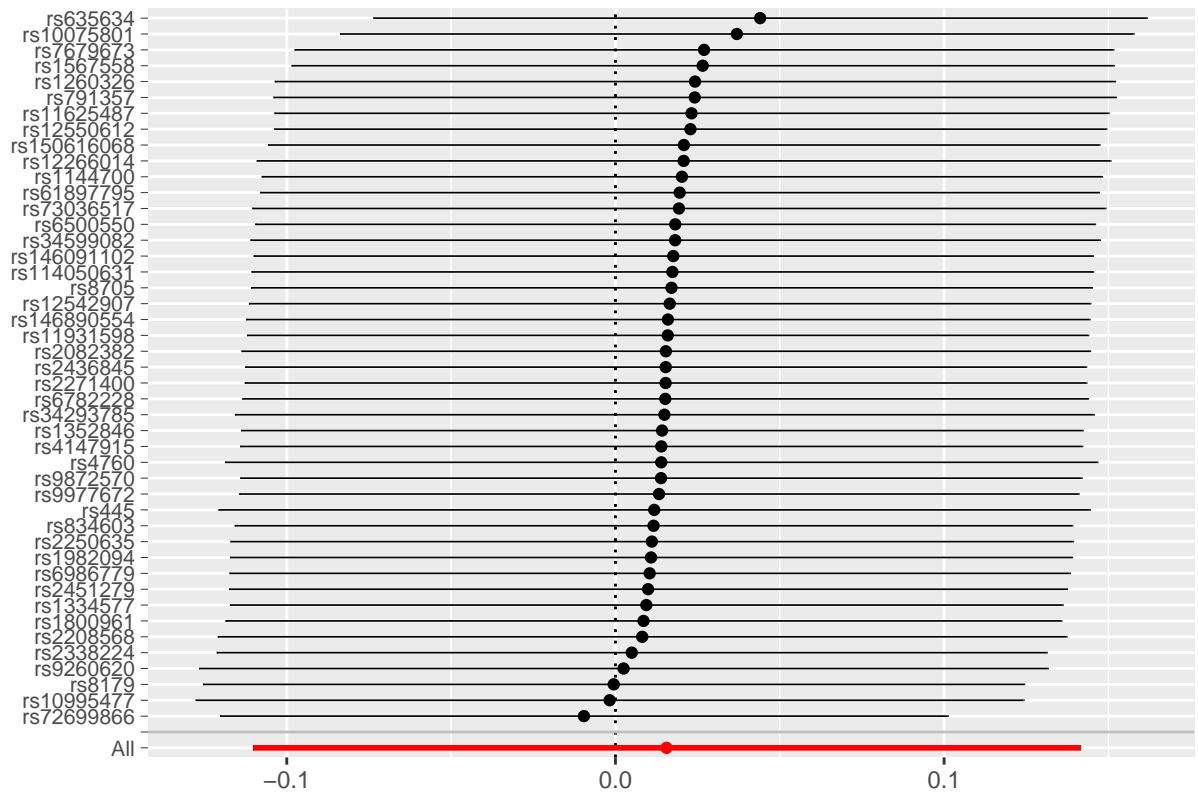


##

\$'nIt4Xv.ieu-a-1126'

Warning: Removed 1 rows containing missing values (geom_errorbarh).

Warning: Removed 1 rows containing missing values (geom_point).

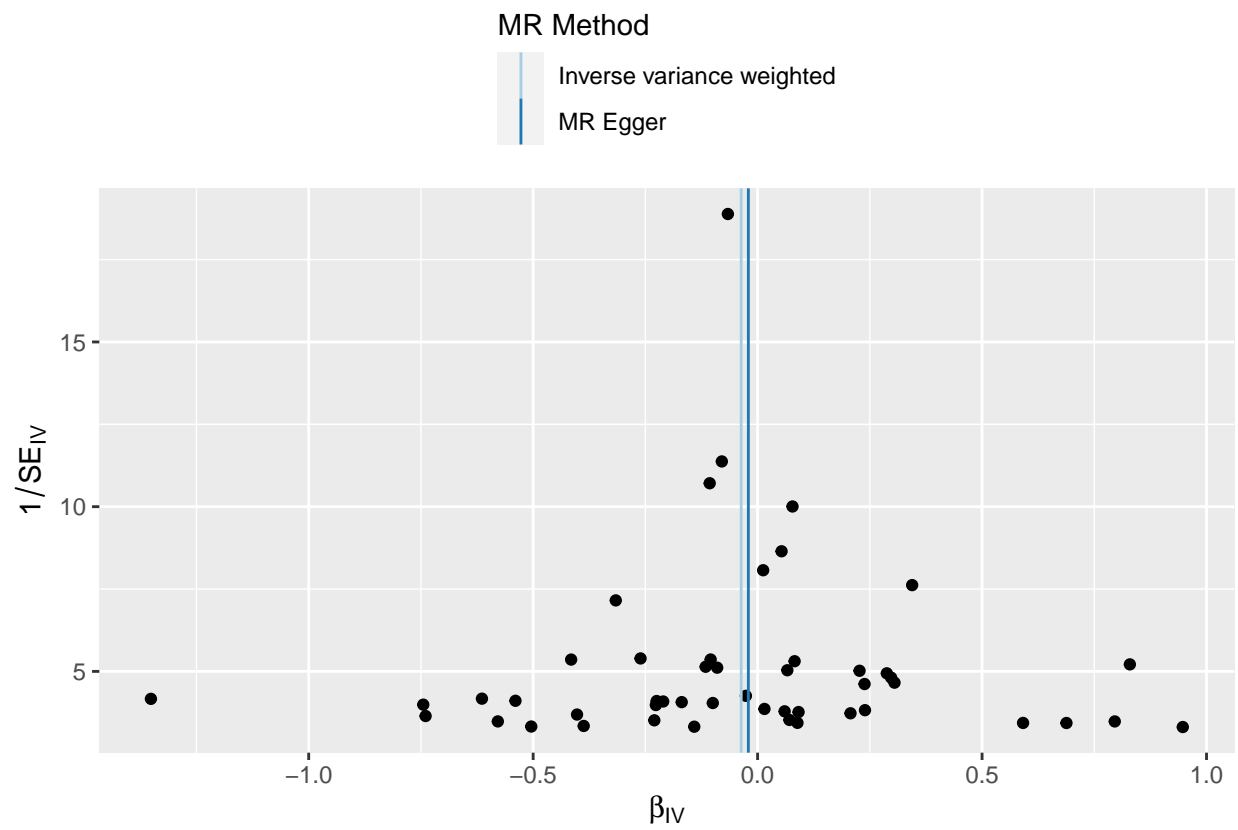


```
##
## attr(,"split_type")
## [1] "data.frame"
## attr(,"split_labels")
##   id.exposure id.outcome
## 1      d8Rvo4 ieu-a-1126
## 2      nIt4Xv ieu-a-1126
```

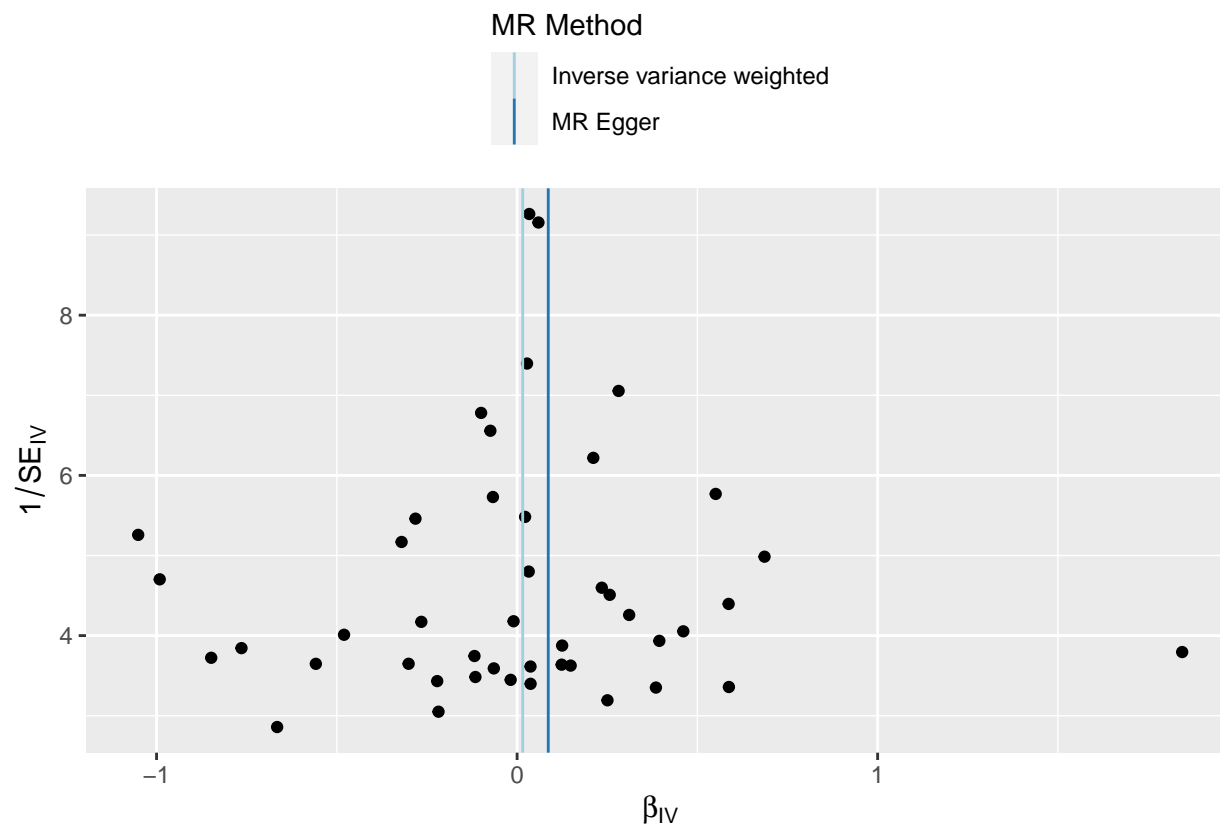
Funnel plot heterogeneity

```
plot4 <- mr_funnel_plot(res_single)
plot4
```

```
## $'d8Rvo4.ieu-a-1126'
```



```
##
## $'nIt4Xv.ieu-a-1126'
```



```
##
## attr("split_type")
## [1] "data.frame"
## attr("split_labels")
##   id.exposure id.outcome
## 1      d8Rvo4 ieu-a-1126
## 2      nIt4Xv ieu-a-1126
```