GSMR

Generalised Summary-data-based Mendelian Randomisaion

Overview

The **gsmr** R-package implements the GSMR (Generalised Summary-data-based Mendelian Randomisation) method to test for putative causal association between a risk factor and a disease using summary-level data from genome-wide association studies (GWAS)[1]. The R package is developed by Zhihong Zhu, Zhili Zheng, Futao Zhang and Jian Yang at Institute for Molecular Bioscience, the University of Queensland. Bug reports or questions: jian.yang@uq.edu.au.

Citation

Zhu, Z. et al. Causal associations between risk factors and common diseases inferred from GWAS summary data. Nature Communications, in press. An early verison of the manuscript is available at bioRxiv, 168674.

Installation

The **gsmr** requires R >= 2.15, you can install it in R by:

```
# gsmr requires the R-package(s)
install.packages(c('survey'));
# install gsmr
install.packages("http://cnsgenomics.com/software/gsmr/static/gsmr_1.0.6.tar.gz",repos=NULL,type="source")
```

The gsmr source codes are available in gsmr_1.0.6.tar.gz. Sample data is available in test_data.zip.

This online document has been integrated in the gsmr R-package, we can check that by the standard “?function_name” command in R.

Update log

V1.0.5 (gmr_1.0.5.tar.gz PDF, 13 Dec. 2017): Improved the approximation of the sampling covariance matrix.

V1.0.4 (gsmr_1.0.4.tar.gz PDF, 6 Nov 2017): Add the bi-directional GSMR analysis. The HEIDI-outlier analysis has been integrated in the GSMR analysis by default.

V1.0.3 (gsmr_1.0.3.tar.gz PDF, 12 Oct 2017): Add more example data.

Removed the initial versions (8 Nov 2016).

Tutorial

The GSMR analysis only requires summary-level data from GWAS. Here is an example, where the risk factor (*x*) is LDL cholesterol (LDL-c) and the disease (*y*) is coronary artery disease (CAD). GWAS summary data for both LDL-c and CAD are available in the public domain (Global Lipids Genetics Consortium et al. 2013, Nature Genetics; Nikpay, M. et al. 2015, Nature Genetics).

1. Prepare data for GSMR analysis

1.1 Load the GWAS sumamry data

```
library("gsmr")
```

```
## Loading required package: methods
```

```
data("gsmr")
head(gsmr_data)
```

```
##          SNP a1 a2       freq     bzx bzx_se  bzx_pval    bzx_n       bzy
## 1 rs10903129  A  G 0.45001947 -0.0328 0.0037 3.030e-17 169920.0  0.008038
## 2 rs12748152  T  C 0.08087758  0.0499 0.0066 3.209e-12 172987.5  0.013671
## 3 rs11206508  A  G 0.14396988  0.0434 0.0055 2.256e-14 172239.0  0.030222
## 4 rs11206510  C  T 0.19128911 -0.0831 0.0050 2.380e-53 172812.0 -0.074519
## 5 rs10788994  T  C 0.18395430  0.0687 0.0049 8.867e-41 172941.9  0.038267
## 6   rs529787  G  C 0.19713099 -0.0553 0.0052 8.746e-24 161969.0  0.001707
##      bzy_se     bzy_pval  bzy_n
## 1 0.0092442 0.3845651000 184305
## 2 0.0185515 0.4611690000 184305
## 3 0.0141781 0.0330400000 184305
## 4 0.0133438 0.0000000234 184305
## 5 0.0118752 0.0012711000 184305
## 6 0.0135491 0.8997431000 184305
```

```
dim(gsmr_data)
```

```
## [1] 189  12
```

This is the input format for the GSMR analysis below. In this data set, there are 189 near-independent SNPs associated with LDL-c at a genome-wide significance level (i.e. p < 5e-8).

- SNP: the genetic instrument
- a1: effect allele
- a2: the other allele
- freq: frequency of a1
- bzx: the effect size of a1 on risk factor
- bzx_se: standard error of bzx
- bzx_pval: p value for bzx
- bzx_n: per-SNP sample size of GWAS for the risk factor
- bzy: the effect size of a1 on disease
- bzy_se: standard error of bzy
- bzy_pval: p value for bzy
- bzy_n: per-SNP sample size of GWAS for the disease

1.2 Estimate the LD correlation matrix

```
# Save the genetic variants and effect alleles in a text file using R
write.table(gsmr_data[,c(1,2)], "gsmr_example_snps.allele", col.names=F, row.names=F, quote=F)
# Extract the genotype data from a PLINK file using GCTA
gcta64 --bfile gsmr_example --extract gsmr_example_snps.allele --update-ref-allele gsmr_example_snps.allele --recode --out gsmr_example
```

Note: the two steps above guarantee that the LD correlations are calculated based on the effect alleles for the SNP effects.

```
# Estimate LD correlation matrix using R
snp_coeff_id = scan("gsmr_example.xmat.gz", what="", nlines=1)
snp_coeff = read.table("gsmr_example.xmat.gz", header=F, skip=2)
```

```
# Take the same SNPs with same order
snp_id = Reduce(intersect, list(gsmr_data$SNP, snp_coeff_id))
gsmr_data = gsmr_data[match(snp_id, gsmr_data$SNP),]
snp_order = match(snp_id, snp_coeff_id)
snp_coeff_id = snp_coeff_id[snp_order]
snp_coeff = snp_coeff[, snp_order]

# Calculate LD correlation matrix
ldrho = cor(snp_coeff)

# Check the size of the correlation matrix and double-check if the order of the SNPs in the LD correlation matrix is consistent with that in the GWAS summary data
colnames(ldrho) = rownames(ldrho) = snp_coeff_id
```

```
dim(ldrho)
```

```
## [1] 189 189
```

```
# Show the first 5 rows and columns of the matrix
ldrho[1:5,1:5]
```

```
##              rs10903129    rs12748152   rs11206508  rs11206510
## rs10903129  1.000000000 -0.0045378845  0.008066621 -0.01372112
## rs12748152 -0.004537884  1.0000000000 -0.006687181  0.00445927
## rs11206508  0.008066621 -0.0066871806  1.000000000 -0.21125757
## rs11206510 -0.013721120  0.0044592696 -0.211257567  1.00000000
## rs10788994 -0.023444710  0.0003629201  0.051259343 -0.18427062
##               rs10788994
## rs10903129 -0.0234447102
## rs12748152  0.0003629201
## rs11206508  0.0512593434
## rs11206510 -0.1842706205
## rs10788994  1.0000000000
```

Note: all the analyses implemented in this R-package only require the summary data (e.g. “gsmr_data”) and the LD correlation matrix (e.g. “ldrho”) listed above.

2. Standardization

This is an optional process. If the risk factor was not standardised in GWAS, the effect sizes can be scaled using the method below. Note that this process requires allele frequencies, z-statistics and sample size. After the scaling, bzx is interpreted as the per-allele effect of a SNP on the exposure in standard deviation units.

```
snpfreq = gsmr_data$freq             # minor allele frequencies of SNPs
bzx = gsmr_data$bzx     # effects of instruments on risk factor
bzx_se = gsmr_data$bzx_se       # standard errors of bzx
bzx_n = gsmr_data$bzx_n          # sample size for GWAS of the risk factor
std_zx = std_effect(snpfreq, bzx, bzx_se, bzx_n)    # perform standardize
gsmr_data$std_bzx = std_zx$b    # standardized bzx
gsmr_data$std_bzx_se = std_zx$se    # standardized bzx_se
head(gsmr_data)
```

```
##          SNP a1 a2       freq     bzx bzx_se  bzx_pval    bzx_n       bzy
## 1 rs10903129  A  G 0.45001947 -0.0328 0.0037 3.030e-17 169920.0  0.008038
## 2 rs12748152  T  C 0.08087758  0.0499 0.0066 3.209e-12 172987.5  0.013671
## 3 rs11206508  A  G 0.14396988  0.0434 0.0055 2.256e-14 172239.0  0.030222
## 4 rs11206510  C  T 0.19128911 -0.0831 0.0050 2.380e-53 172812.0 -0.074519
## 5 rs10788994  T  C 0.18395430  0.0687 0.0049 8.867e-41 172941.9  0.038267
## 6   rs529787  G  C 0.19713099 -0.0553 0.0052 8.746e-24 161969.0  0.001707
##      bzy_se     bzy_pval  bzy_n     std_bzx  std_bzx_se
## 1 0.0092442 0.3845651000 184305 -0.03055942 0.003447252
## 2 0.0185515 0.4611690000 184305  0.04713698 0.006234550
## 3 0.0141781 0.0330400000 184305  0.03829018 0.004852442
## 4 0.0133438 0.0000000234 184305 -0.07181919 0.004321251
## 5 0.0118752 0.0012711000 184305  0.06149455 0.004386074
## 6 0.0135491 0.8997431000 184305 -0.04695042 0.004414868
```

3. GSMR analysis

This is the main analysis of this R-package. It uses SNPs associated with the risk factor (e.g. at p < 5e-8) as the instruments to test for putative causal effect of the risk factor on the disease. The analysis involves a step that uses the HEIDI-outlier approach to remove SNPs that have effects on both the risk factor and the diseae because of pleiotropy.

```
bzx = gsmr_data$std_bzx    # SNP effects on risk factor
bzx_se = gsmr_data$std_bzx_se    # standard errors of bzx
bzx_pval = gsmr_data$bzx_pval   # p-values for bzx
bzy = gsmr_data$bzy    # SNP effects on disease
bzy_se = gsmr_data$bzy_se    # standard errors of bzy
bzy_pval = gsmr_data$bzy_pval    # p-values for bzy
n_ref = 7703    # Sample size of the reference sample
gwas_thresh = 5e-8    # GWAS threshold to select SNPs as the instruments for the GSMR analysis
heidi_outlier_thresh = 0.01    # HEIDI-outlier threshold
nsnps_thresh = 10   # the minimum number of instruments required for the GSMR analysis
heidi_outlier_flag = T    # flag for HEIDI-outlier analysis
ld_fdr_thresh = 0.05   # FDR threshold to remove the chance correlations between SNP instruments
gsmr_results = gsmr(bzx, bzx_se, bzx_pval, bzy, bzy_se, ldrho, snp_coeff_id, n_ref, heidi_outlier_flag, gwas_thresh, heidi_outlier_thresh, nsnps_
thresh, ld_fdr_thresh)    # GSMR analysis
cat("Effect of exposure on outcome: ",gsmr_results$bxy)
```

```
## Effect of exposure on outcome:  0.4050761
```

```
cat("Standard error of bxy: ",gsmr_results$bxy_se)
```

```
## Standard error of bxy:  0.0229115
```

```
cat("P-value of bxy: ", gsmr_results$bxy_pval)
```

```
## P-value of bxy:  5.975967e-70
```

```
cat("Used index to GSMR analysis: ", gsmr_results$used_index[1:5], "...")
```

```
## Used index to GSMR analysis:  1 2 3 5 6 ...
```

4. HEIDI-outlier analysis

The estimate of causal effect of risk factor on disease can be biased by pleiotropy (Zhu et al. 2017 bioRxiv). This is an analysis to detect and eliminate from the analysis instruments that show significant pleiotropic effects on both risk factor and disease. The HEIDI-outlier analysis requires bzx (effect of genetic instrument on risk factor), bzx_se (standard error of bzx), bzx_pval (p-value of bzx), bzy (effect of genetic instrument on disease), bzy_se (standard error of bzy) and ldrho (LD matrix of instruments). Note that LD matrix can be estimated from a reference sample with individual-level genotype data.

The HEIDI-outlier analysis has been integrated in the GSMR analysis above (with the heidi_outlier_flag and heidi_outlier_thresh flags). It can also be performed separately following the example below.

```
filtered_index = heidi_outlier(bzx, bzx_se, bzx_pval, bzy, bzy_se, ldrho, snp_coeff_id, n_ref, gwas_thresh, heidi_outlier_thresh, nsnps_thresh, l
d_fdr_thresh) # perform HEIDI-outlier analysis
filtered_gsmr_data = gsmr_data[filtered_index,]   # select data passed HEIDI-outlier filtering

filtered_snp_id = snp_coeff_id[filtered_index]   # select SNPs that passed HEIDI-outlier filtering

dim(filtered_gsmr_data)
```

```
## [1] 138  14
```

```
# number of SNPs in the gmsr_data with bzx_pval < 5e-8
dim(gsmr_data[gsmr_data$bzx_pval < 5e-8, ])
```

```
## [1] 151  14
```

In the example above, 13 SNPs are filtered out by HEIDI-outlier.

5. Bi-directional GSMR analysis

The script below runs bi-directional GSMR analyses, i.e. a forward-GSMR analysis as described above and a reverse-GSMR analysis that uses SNPs associated with the disease (e.g. at p < 5e-8) as the instruments to test for putative causal effect of the disease on the risk factor.

```
gsmr_results = bi_gsmr(bzx, bzx_se, bzx_pval, bzy, bzy_se, bzy_pval, ldrho, snp_coeff_id, n_ref, heidi_outlier_flag, gwas_thresh, heidi_outlier_t
hresh, ld_fdr_thresh)    # GSMR analysis
cat("Effect of risk factor on disease: ",gsmr_results$forward_bxy)
```

```
## Effect of risk factor on disease:  0.4050761
```

```
cat("Standard error of bxy from the forward-GSMR analysis: ",gsmr_results$forward_bxy_se)
```

```
## Standard error of bxy from the forward-GSMR analysis:  0.0229115
```

```
cat("P-value of bxy from the forward-GSMR analysis: ", gsmr_results$forward_bxy_pval)
```

```
## P-value of bxy from the forward-GSMR analysis:  5.975967e-70
```

```
cat("Effect of disease on risk factor: ",gsmr_results$reverse_bxy)
```

```
## Effect of disease on risk factor:  -0.02376614
```

```
cat("Standard error of bxy from the reverse-GSMR analysis: ",gsmr_results$reverse_bxy_se)
```

```
## Standard error of bxy from the reverse-GSMR analysis:  0.00958462
```

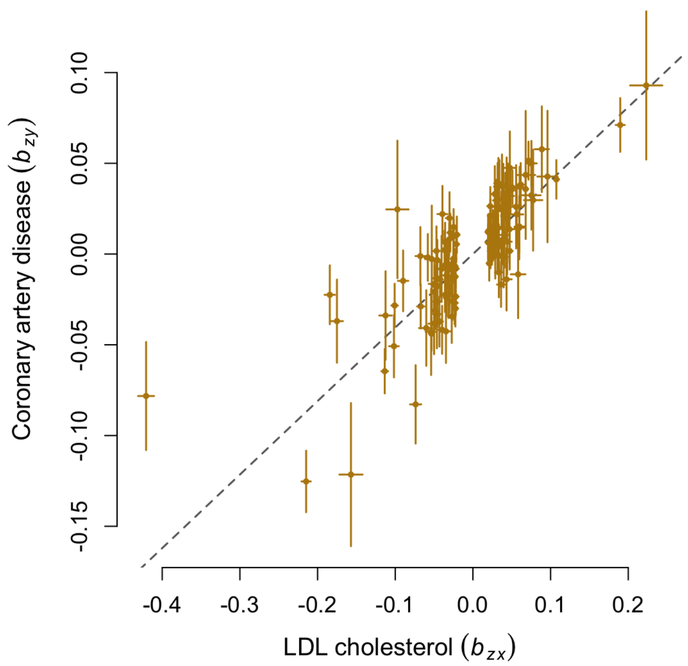
```
cat("P-value of bxy from the reverse-GSMR analysis: ", gsmr_results$reverse_bxy_pval)
```

```
## P-value of bxy from the reverse-GSMR analysis:  0.01315254
```

6. Visulization

```
effect_col = colors()[75]
vals = c(bzx[filtered_index]-bzx_se[filtered_index], bzx[filtered_index]+bzx_se[filtered_index])
xmin = min(vals); xmax = max(vals)
vals = c(bzy[filtered_index]-bzy_se[filtered_index], bzy[filtered_index]+bzy_se[filtered_index])
ymin = min(vals); ymax = max(vals)
par(mar=c(5,5,4,2))
plot(bzx[filtered_index], bzy[filtered_index], pch=20, cex=0.8, bty="n", cex.axis=1.1, cex.lab=1.2,
        col=effect_col, xlim=c(xmin, xmax), ylim=c(ymin, ymax),
        xlab=expression(LDL~cholesterol~(italic(b[zx]))),
        ylab=expression(Coronary~artery~disease~(italic(b[zy]))))
abline(0, gsmr_results$forward_bxy, lwd=1.5, lty=2, col="dim grey")

nsnps = length(bzx[filtered_index])
for( i in 1:nsnps ) {
    # x axis
    xstart = bzx[filtered_index [i]] - bzx_se[filtered_index[i]]; xend = bzx[filtered_index[i]] + bzx_se[filtered_index[i]]
    ystart = bzy[filtered_index[i]]; yend = bzy[filtered_index[i]]
    segments(xstart, ystart, xend, yend, lwd=1.5, col=effect_col)
    # y axis
    xstart = bzx[filtered_index[i]]; xend = bzx[filtered_index[i]]
    ystart = bzy[filtered_index[i]] - bzy_se[filtered_index[i]]; yend = bzy[filtered_index[i]] + bzy_se[filtered_index[i]]
    segments(xstart, ystart, xend, yend, lwd=1.5, col=effect_col)
}
```



Package Document

bi_gsmr

> Bi-directional GSMR analysis is composed of a forward-GSMR analysis and a reverse-GSMR analysis that uses SNPs associated with the disease (e.g. at < 5e-8) as the instruments to test for putative causal effect of the disease on the risk factor.

Usage

```
bi_gsmr(bzx, bzx_se, bzx_pval, bzy, bzy_se, bzy_pval, ldrho, snpid, heidi_outlier_flag=T, gwas_thresh=5e-8, heidi_outlier_thresh=0.01, nsnps_thresh
=10)
```

Arguments

`bzx`	vector, SNP effects on risk factor
`bzx_se`	vector, standard errors of bzx
`bzx_pval`	vector, p values for bzx
`bzy`	vector, SNP effects on disease

`bzy_se`	vector, standard errors of bzy
`bzy_pval`	vector, p values for bzy
`ldrho`	LD correlation matrix of the SNPs
`snpid`	genetic instruments
`n_ref`	sample size of the reference sample
`heidi_outlier_flag`	flag for HEIDI-outlier analysis
`gwas_thresh`	threshold p-value to select instruments from GWAS for risk factor
`heidi_outlier_thresh`	HEIDI-outlier threshold
`nsnps_thresh`	the minimum number of instruments required for the GSMR analysis (we do not recommend users to set this number smaller than 10)
`ld_fdr_thresh`	FDR threshold to remove the chance correlations between SNP instruments

Value

Estimate of causative effect of risk factor on disease (forward_bxy), the corresponding standard error (forward_bxy_se), p-value (forward_bxy_pval) and SNP index (forward_index), and estimate of causative effect of disease on risk factor (reverse_bxy), the corresponding standard error (reverse_bxy_se), p-value (reverse_bxy_pval) and SNP index (reverse_index).

Examples

```
data("gsmr")
gsmr_result = bi_gsmr(gsmr_data$bzx, gsmr_data$bzx_se, gsmr_data$bzx_pval, gsmr_data$bzy, gsmr_data$bzy_se, gsmr_data$bzy_pval, ldrho, gsmr_data$SNP, n_ref, T, 5e-8, 0.01, 10, 0.05)
```

gsmr

> GSMR (Generalised Summary-data-based Mendelian Randomisation) is a flexible and powerful approach that utilises multiple genetic instruments to test for causal association between a risk factor and disease using summary-level data from independent genome-wide association studies.

Usage

```
gsmr(bzx, bzx_se, bzx_pval, bzy, bzy_se, ldrho, snpid, heidi_outlier_flag=T, gwas_thresh=5e-8, heidi_outlier_thresh=0.01, nsnps_thresh=10)
```

Arguments

`bzx`	vector, SNP effects on risk factor
`bzx_se`	vector, standard errors of bzx
`bzx_pval`	vector, p values for bzx
`bzy`	vector, SNP effects on disease
`bzy_se`	vector, standard errors of bzy
`ldrho`	LD correlation matrix of the SNPs
`snpid`	genetic instruments
`n_ref`	sample size of the reference sample
`heidi_outlier_flag`	flag for HEIDI-outlier analysis
`gwas_thresh`	threshold p-value to select instruments from GWAS for risk factor
`heidi_outlier_thresh`	HEIDI-outlier threshold
`nsnps_thresh`	the minimum number of instruments required for the GSMR analysis (we do not recommend users to set this number smaller than 10)
`ld_fdr_thresh`	FDR threshold to remove the chance correlations between SNP instruments

Value

Estimate of causative effect of risk factor on disease (bxy), the corresponding standard error (bxy_se), p-value (bxy_pval) and SNP index (used_index).

Examples

```
data("gsmr")
gsmr_result = gsmr(gsmr_data$bzx, gsmr_data$bzx_se, gsmr_data$bzx_pval, gsmr_data$bzy, gsmr_data$bzy_se, ldrho, gsmr_data$SNP, n_ref, T, 5e-8, 0.
01, 10, 0.05)
```

heidi_outlier

> An analysis to detect and eliminate from the analysis instruments that show significant pleiotropic effects on both risk factor and disease

Usage

```
heidi_outlier(bzx, bzx_se, bzx_pval, bzy, bzy_se, ldrho, snpid, n_ref, gwas_thresh=5e-8, heidi_outlier_thresh=0.01, nsnps_thresh=10, ld_fdr_thresh=
0.05)
```

Arguments

`bzx`	vector, SNP effects on risk factor
`bzx_se`	vector, standard errors of bzx
`bzx_pval`	vector, p values for bzx
`bzy`	vector, SNP effects on disease
`bzy_se`	vector, standard errors of bzy
`ldrho`	LD correlation matrix of the SNPs
`snpid`	genetic instruments
`n_ref`	sample size of the reference sample
`gwas_thresh`	threshold p-value to select instruments from GWAS for risk factor
`heidi_outlier_thresh`	threshold p-value to remove pleiotropic outliers (the default value is 0.01)
`nsnps_thresh`	the minimum number of instruments required for the GSMR analysis (we do not recommend users to set this number smaller than 10)
`ld_fdr_thresh`	FDR threshold to remove the chance correlations between SNP instruments

Value

Retained index of genetic instruments

Examples

```
data("gsmr")
filtered_index = heidi_outlier(gsmr_data$bzx, gsmr_data$bzx_se, gsmr_data$bzx_pval, gsmr_data$bzy, gsmr_data$bzy_se, ldrho, gsmr_data$SNP, n_ref,
5e-8, 0.01, 10, 0.05)
```

std_effect

> Standardization of SNP effect and its standard error using z-statistic, allele frequency and sample size

Usage

```
std_effect(snp_freq, b, se, n)
```

Arguments

`snp_freq`	vector, allele frequencies
`b`	vector, SNP effects on risk factor
`se`	vector, standard errors of b
`n`	vector, per-SNP sample sizes for GWAS of the risk factor

Value

Standardised effect (b) and standard error (se)

Examples

```
data("gsmr")
std_effects = std_effect(gsmr_data$freq, gsmr_data$bzx, gsmr_data$bzx_se, gsmr_data$bzx_n)
```