CARMA Tutorial

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Introduction

This document describes a complete walk through the usage of the package 'CARMA' with an application to computing the posterior inclusion probability (PIP) of variants at loci of interest. In this document, we will illustrate:

- Summary statistics and in-sample linkage disequilibrium (LD) matrix.
- Summary statistics and LD matrix extracted from external panels.

Environment preparation

Install the package 'CARMA'.

```
devtools::install_github("ZikunY/CARMA")
library("CARMA")
```

Create a folder 'CARMA', then download the example datasets on OSF through command wget.

```
mkdir CARMA
cd CARMA
##### Download and save the demo data in folder `CARMA'
wget -O Sample_data.tar.gz https://osf.io/5gqz8/download
##### or download the file from https://osf.io/4t2bz/
tar -zxf Sample_data.tar.gz
```

Install several packages needed to run CARMA.

```
pkgs = c("data.table", "magrittr", "dplyr", "devtools","R.utlis")
pkgs.na = pkgs[!pkgs %in% installed.packages()[, "Package"]]
if (length(pkgs.na) > 0) {
  install.packages(pkgs.na)
}
if (!"CARMA" %in% installed.packages()[, "Package"]) {
  devtools::install_github("ZikunY/CARMA")
}
```

Implementation on M1 Mac

If users encounter issues while installing CARMA on an M1 Mac, it may be due to the failure of locating the GSL library or other libraries during the installation process. This document aims to provide a solution to this problem by modifying the 'PKG_CPPFLAGS' environment variable in the '~/.R/Makevars file'. This file should be created if it doesn't exist. The appropriate lines need to be added to the PKG_CPPFLAGS variable to ensure the GSL library is correctly located.

Setting PKG_LIBS

Open the '~/.R/Makevars' file in a text editor, and add the following line to set the PKG_CPPFLAGS environment variable:

- PKG_CPPFLAGS=-I/opt/homebrew/Cellar/gsl/2.7.1/include/

The pathway above corresponds to the GSL header files' location for GSL version 2.7.1. Depending on the user's system, this pathway may vary.

Setting PKG_LIBS

Next, add the necessary lines to the ~/.R/Makevars file to specify the GSL library's location and other required libraries:

- PKG_LIBS=-L/opt/homebrew/lib -lgsl -lgslcblas -lm

The above line tells the linker (ld) to search for the GSL library (libgsl) and GSL CBLAS (libgslcblas) in the Homebrew installation directory (/opt/homebrew/lib). The -lm flag links the math library.

Important Note

The pathway for the GSL library and related libraries may differ depending on the specific GSL version or installation location. Users should verify the correct pathway on their system and modify the PKG_CPPFLAGS and PKG_LIBS accordingly.

Error when loading CARMA

Several users have reported encountering an error when loading CARMA. To address this issue, **@hlnicholls** from GitHub provided a solution that involves checking the Intel MLK installation instructions available at https://csantill.github.io/RPerformanceWBLAS/. Specifically, running the following line after installation resolved the error:

```
sudo apt-get install libopenblas-base libatlas3-base
```

Additionally, **@hbliu** from GitHub suggested an alternative fix. This approach involves installing Intel MLK via Conda and then changing BLAS/LAPACK using LD_PRELOAD when starting R.

```
conda install -c anaconda mkl
LD_PRELOAD=pathto/anaconda3/lib/libmkl_rt.so R
devtools::install_github("ZikunY/CARMA")
```

We extend our special thanks to **@hlnicholls** and **@hbliu** for their valuable contributions and assistance in resolving this issue! Your support is greatly appreciated.

Individual level data

Simulating data

We simulate individual level data for the purpose of this demonstration. We use the R package 'sim1000G' (Dimitromanolakis et al. 2019) to simulate genotypes based on the 1000 Genomes Project data (phase 3, European population). The phenotype is simulated through

$$\boldsymbol{y} = \boldsymbol{X}\boldsymbol{\beta} + \boldsymbol{\epsilon},$$

where $\boldsymbol{X}$ are the genotypes, $\boldsymbol{\beta}$ is a sparse coefficient vector such as $\beta_i \neq 0$ if the ith SNP is a causal SNP, and $\boldsymbol{\epsilon}$ is the standard Gaussian error. Causal variants are selected according to a probability proportional to the linear predictor $\boldsymbol{w}_i'\boldsymbol{\theta}$, where $\boldsymbol{w}_i$ is the vector of annotations associated with the ith SNP and $\boldsymbol{\theta}$ is the coefficients vector of the annotations.

Example at locus chr1: 200,937,832-201,937,832

In this section, we use the simulated data based on the locus chr1:200,937,832-201,937,832. We computed the summary statistics (Z-scores) and the in-sample LD matrix. The three causal SNPs are highlighted in the plot below (the **287**th, **1275**th, and **2572**th SNPs at the locus; the left, middle, right red points respectively).

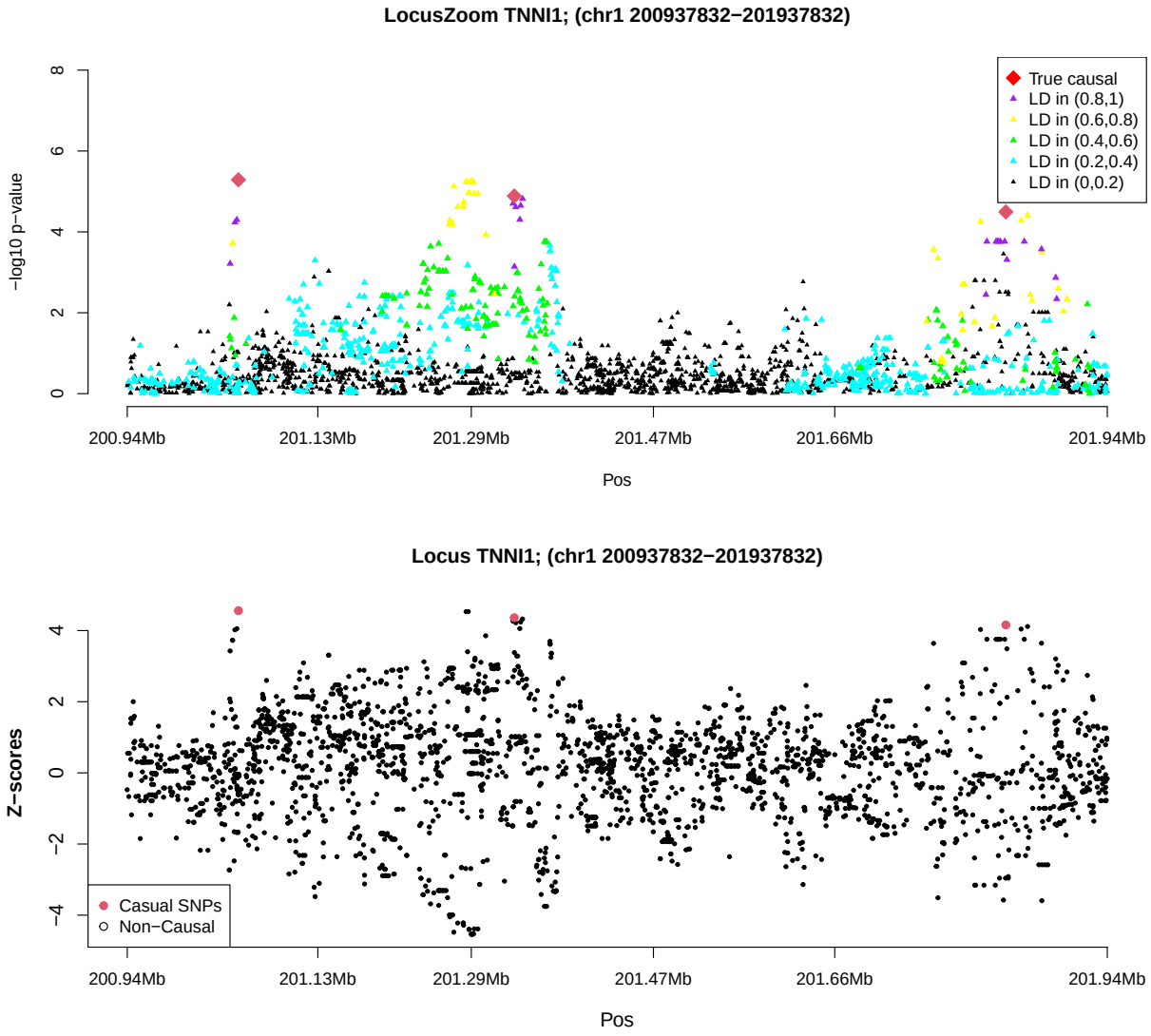


Figure 1: LocusZoom and Z-score plots for locus chr1:200,937,832-201,937,832

As shown in Figure 1, one of the causal SNPs has few highly correlated SNPs and larger Z-scores, whereas the other two SNPs are highly correlated to the surrounding SNPs with similar Z-scores.

Running CARMA without annotations. We run CARMA without annotations first. The input format of CARMA is the list class. We use the "CARMA_fixed_sigma'' function in the package. As recommended in the paper, we choose the dimensional hyperparameter $\eta = 1$, which can be interpreted as the prior expectation of the number of causal SNPs resided in the testing locus. Notice that without annotations, all SNPs have identical prior probabilities of being causal generated by the Poisson prior distribution, which assigns prior probability on the model size and provides false discovery control.

```
library(data.table)
library(magrittr)
library(dplyr)
library(devtools)
library(R.utils)
##### setting up the working directory or the wd where the data are stored
setwd('CARMA')
###### load the GWAS summary statistics
sumstat<- fread(file = "Sample_data/sumstats_chr1_200937832_201937832.txt.gz",
                          sep = "\t", header = T, check.names = F, data.table = F,
                          stringsAsFactors = F)
###### load the pair-wise LD matrix (assuming the variants are sorted in the same order
###### as the variants in sumstat file)
ld =  fread(file = "Sample_data/sumstats_chr1_200937832_201937832_ld.txt.gz",
                       sep = "\t", header = F, check.names = F, data.table = F,
                       stringsAsFactors = F)

print(head(sumstat))
```

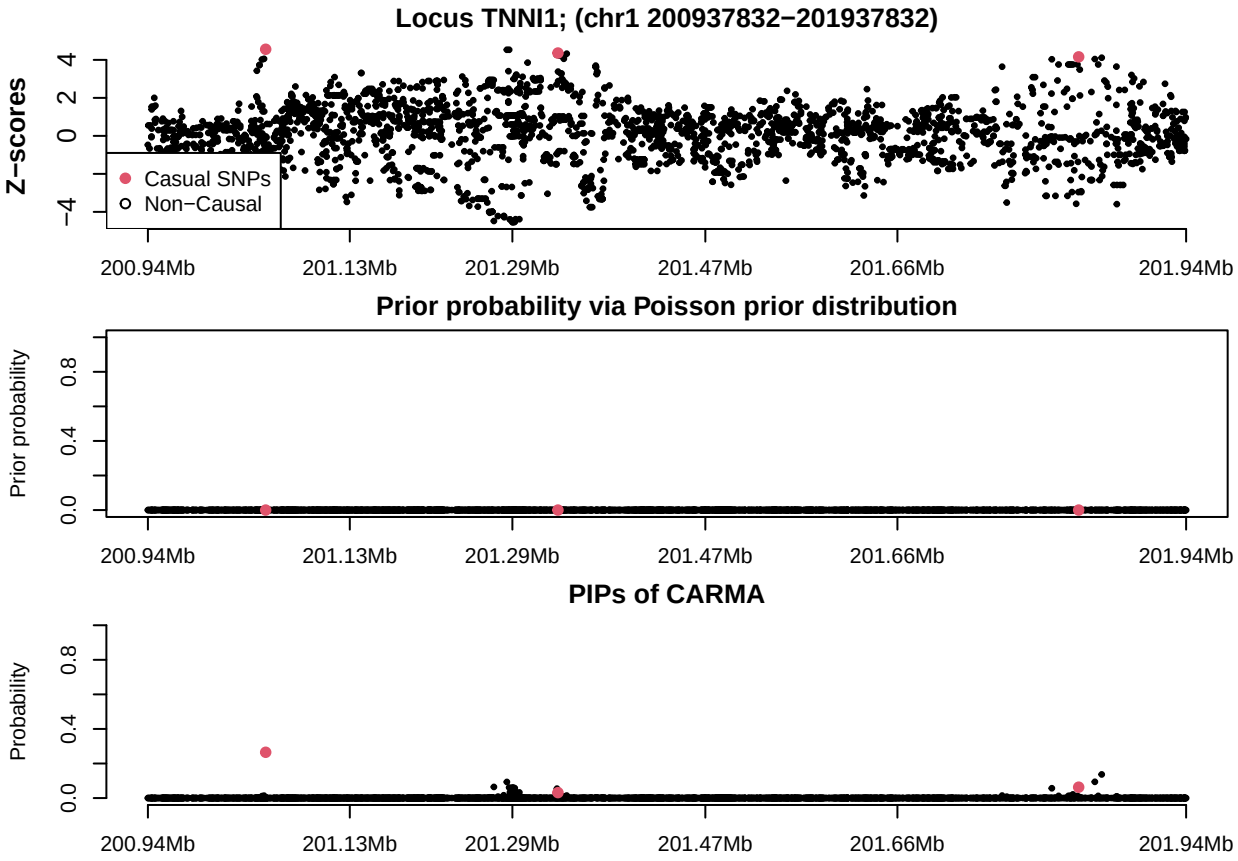
The input data 'sumstat' are typical results containing summary statistics, such as

```
##                 ID CHR       POS Ref Alt        SNP     N  MAF      Z Pval
## 1 1:200938029:G:A   1 200938029   G   A rs10494829 10000 0.26 -0.475 1.37
## 2 1:200938474:G:T   1 200938474   G   T  rs4915210 10000 0.29  0.550 0.58
## 3 1:200939642:C:T   1 200939642   C   T  rs3208703 10000 0.14 -0.666 1.49
## 4 1:200940180:G:A   1 200940180   G   A  rs3198583 10000 0.29  0.550 0.58
## 5 1:200941423:C:A   1 200941423   C   A rs56368827 10000 0.26 -0.071 1.06
## 6 1:200941549:T:C   1 200941549   T   C   rs296570 10000 0.92 -0.031 1.02
```

Next, we run CARMA with the input summary statistics Z and the LD matrix with the hyperparameter $\eta = 1$ as the default setting. Notice that given that the LD matrix is in-sample, we do not turn on the outlier detection 'outlier.switch=F'.

```
z.list<-list()
ld.list<-list()
lambda.list<-list()
z.list[[1]]<-sumstat$Z
ld.list[[1]]<-as.matrix(ld)
lambda.list[[1]]<-1
CARMA.results<-CARMA_fixed_sigma(z.list,ld.list,lambda.list=lambda.list,
                                 outlier.switch=F)
###### Posterior inclusion probability (PIP) and credible set (CS)
sumstat.result = sumstat %>% mutate(PIP = CARMA.results[[1]]$PIPs, CS = 0)
if(length(CARMA.results[[1]]$`Credible set`[[2]])!=0){
  for(l in 1:length(CARMA.results[[1]]$`Credible set`[[2]])){
    sumstat.result$CS[CARMA.results[[1]]$`Credible set`[[2]][[l]]]=l
  }
}
###### write the GWAS summary statistics with PIP and CS
fwrite(x = sumstat.result,
       file = "Sample_data/sumstats_chr1_200937832_201937832_carma.txt.gz",
       sep = "\t", quote = F, na = "NA", row.names = F, col.names = T,
       compress = "gzip")
```

We can check the results.




```
##       SNPs.index Causal.status Z.scores  PIPs
## 287          287   True causal      4.6 0.265
## 2603        2603    Non-causal      4.1 0.137
## 2591        2591    Non-causal      4.0 0.094
## 1095        1095    Non-causal      4.5 0.093
## 1057        1057    Non-causal     -4.5 0.064
## 2572        2572   True causal      4.2 0.063
## 1105        1105    Non-causal      4.5 0.061
## 1120        1120    Non-causal     -4.5 0.060
## 1125        1125    Non-causal     -4.5 0.060
## 1134        1134    Non-causal     -4.5 0.058
## 2530        2530    Non-causal      4.0 0.057
## 1272        1272    Non-causal      4.4 0.055
## 1141        1141    Non-causal     -4.4 0.037
## 1110        1110    Non-causal     -4.4 0.034
## 1158        1158    Non-causal     -4.4 0.033
## 1121        1121    Non-causal     -4.5 0.032
## 1131        1131    Non-causal     -4.5 0.032
## 1275        1275   True causal      4.4 0.031
## 2562        2562    Non-causal      3.8 0.021
## 1281        1281    Non-causal      4.2 0.018
```

We can observe that the 287th SNP (the causal SNP at left) received a larger PIP value to the surrounding SNPs. On the other hand, the other two causal SNPs, which are highly correlated to surrounding SNPs with similar Z-scores, shared the PIPs with the highly correlated SNPs. We can also check the credible sets and credible models.

```
CARMA.results[[1]]$`Credible set`[[2]]
```

```
## list()
```

```
CARMA.results[[1]]$`Credible model`[[3]]
```

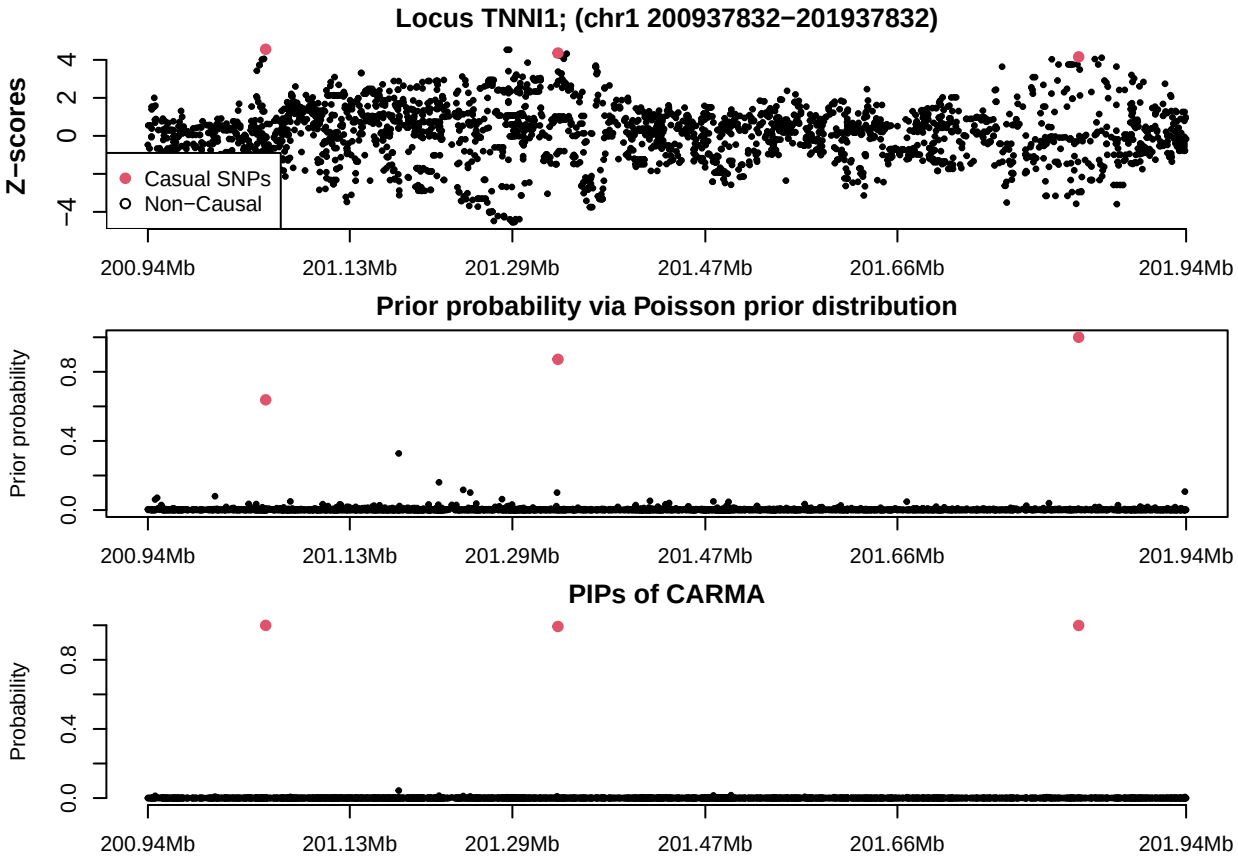
```
##  [1]  287 1120 1121 1125 1131 1095 1105 2603 1134 1057 2591 2530 2572 1109 1110
## [16] 1141 1158 1272 1273 1275 1304 1086 1268 1298 1063 1082 1084 1281 1294  269
## [31] 1043 2624  257 2539 2550 2552 2557 2562 2569 2597
```

Due to relatively weak signal strength, none of the signals have enough PIPs to formulate credible sets. On the other hand, credible model still identified 22 candidate SNPs, which include all three true causal SNPs.

Running CARMA with annotations We can include functional annotations into CARMA:

```
###### load the functional annotations for the variants included in GWAS summary
###### statistics (assuming the variants are sorted in the same order as the
###### variants in sumstat file)
annot=fread(file = "Sample_data/sumstats_chr1_200937832_201937832_annotations.txt.gz",
            sep="\t", header = T, check.names = F, data.table = F,
            stringsAsFactors = F)
###### z.list and ld.list stay the same with the previous setting,
###### and we add annotations this time.
annot.list<-list()
annot.list[[1]]<-annot
CARMA.results<-CARMA_fixed_sigma(z.list,ld.list,lambda.list=lambda.list,w.list=annot.list,
                                 outlier.switch=F)
###### Posterior inclusion probability (PIP) and credible set (CS)
sumstat.result = sumstat %>% mutate(PIP = CARMA.results[[1]]$PIPs, CS = 0)
if(length(CARMA.results[[1]]$`Credible set`[[2]])!=0){
  for(l in 1:length(CARMA.results[[1]]$`Credible set`[[2]])){
    sumstat.result$CS[CARMA.results[[1]]$`Credible set`[[2]][[l]]]=l
  }
}
###### write the GWAS summary statistics with PIP and CS
fwrite(x = sumstat.result,
       file = "Sample_data/sumstats_chr1_200937832_201937832_carma_annot.txt.gz",
       sep = "\t", quote = F, na = "NA", row.names = F, col.names = T,
       compress = "gzip")
```

We can first check the resulting PIPs. This time, we also include the prior probability of a variant being causal estimated by CARMA.




```
##       SNPs.index Causal.status Z.scores   PIPs
## 287          287   True causal     4.56 0.9996
## 2572        2572   True causal     4.16 0.9988
## 1275        1275   True causal     4.36 0.9930
## 724          724    Non-causal     0.38 0.0436
## 1827        1827    Non-causal    -2.58 0.0166
## 1709        1709    Non-causal     0.70 0.0152
## 898          898    Non-causal     0.47 0.0138
## 16            16    Non-causal     0.69 0.0129
## 967          967    Non-causal     1.14 0.0118
## 1273        1273    Non-causal     4.36 0.0101
## 985          985    Non-causal     1.22 0.0094
## 142          142    Non-causal    -0.28 0.0083
## 2018        2018    Non-causal    -0.50 0.0072
## 363          363    Non-causal    -0.25 0.0066
## 2796        2796    Non-causal    -0.77 0.0062
## 2083        2083    Non-causal     1.68 0.0051
## 2521        2521    Non-causal    -3.16 0.0051
## 2249        2249    Non-causal    -1.23 0.0049
## 1095        1095    Non-causal     4.53 0.0046
## 2523        2523    Non-causal    -1.63 0.0044
```

As observed from the figure above, the inclusion of annotations helps CARMA distinguish the true causal variants from the highly correlated SNPs, such as the 1275th and 2572th SNP, which in the absence of functional annotations cannot be distinguished from other highly correlated SNPs. Also, the 287th SNP receives a larger PIP this time. We can also examine the credible sets and credible models of CARMA.

```
CARMA.results[[1]]$`Credible set`[[2]]
```

```
## [[1]]
## [1] 287
##
## [[2]]
## [1] 2572
##
## [[3]]
## [1] 1275
```

```
CARMA.results[[1]]$`Credible model`[[3]]
```

```
## [1]  287 1275 2572
```

The number of SNPs in credible models has been reduced significantly. Also, the credible sets identified the three true causals.

Summary statistics with LD matrix extracted from reference panels

Usually, individual level data are not available in meta-analysis GWAS studies. Instead, summary statistics are made available and an external LD matrix is used. These complex meta-analysis settings create inconsistencies between summary statistics and LD values which can lead to biased PIP values.

We use summary statistics from a meta-analysis for Alzheimer's disease (AD) (Jansen et al. 2019). The meta-analysis of AD is based on clinically diagnosed AD and AD-by-proxy with 71,880 cases and 383,378 controls of European ancestry. The clinically diagnosed AD case-control data are from 3 consortia (PGC-ALZ, IGAP, and ADSP), and the AD-by-proxy data are based on 376,113 individuals of European ancestry from UK BioBank (UKBB). We use the LD matrix extracted from the UKBB. For the CARMA model, we include 187 annotations provided by PolyFun plus PolyFun prior causal probability (Weissbrod et al. 2020).

Demonstration with loci ADAMTS4 and CR1

We illustrate CARMA on two loci, ADAMTS4 and CR1 on chromosome 1. We extract data at locus ADAMTS4/CR1, and extract the corresponding LD matrices from the UKBB (provided by PolyFun).

Sample of data at the locus ADAMTS4

```
##       uniqID.a1a2 CHR        BP A1 A2         SNP     Z     P   Nsum   Neff
## 1 1:160656603_A_T   1 160656603  A  T   rs6702441  0.15 0.881 429975 423497
## 2 1:160657127_T_C   1 160657127  T  C rs143426473  0.81 0.417 435185 428660
## 3 1:160657137_G_A   1 160657137  G  A  rs11589131  0.05 0.960 429757 423281
## 4 1:160657197_C_G   1 160657197  C  G  rs10908797  1.75 0.079 377075 375815
## 5 1:160657356_C_T   1 160657356  C  T rs145169682 -0.28 0.776  17477  17477
## 6 1:160658364_G_A   1 160658364  G  A   rs7539434  0.24 0.814 380902 379635
##    dir   EAF     BETA     SE
## 1 ?+-+ 0.370  0.00034 0.0023
## 2 ?+++ 0.034  0.00486 0.0060
## 3 ?+-+ 0.369  0.00011 0.0023
## 4 ??++ 0.097  0.00685 0.0039
## 5 ???- 0.012 -0.01407 0.0495
## 6 ??++ 0.028  0.00163 0.0069
```

Sample of data at the locus CR1

```
##       uniqID.a1a2 CHR        BP A1 A2         SNP      Z    P   Nsum   Neff
## 1 1:207287187_T_C   1 207287187  T  C   rs2808470  0.755 0.45 433909 427395
## 2 1:207288258_C_T   1 207288258  C  T rs147553990 -0.627 0.53 364527 364527
```

```
## 3 1:207288297_T_C   1 207288297  T  C  rs17020983  1.136 0.26 434723 428202
## 4 1:207288309_T_G   1 207288309  T  G  rs79498904  0.879 0.38 364051 364051
## 5 1:207288392_G_A   1 207288392  G  A  rs17020993  1.108 0.27 436498 429961
## 6 1:207288897_T_C   1 207288897  T  C  rs12031629  0.094 0.93  71639  71639
##    dir    EAF    BETA     SE
## 1 ?-++ 0.1940  0.0021 0.0027
## 2 ??-? 0.0033 -0.0127 0.0203
## 3 ?-++ 0.0960  0.0042 0.0037
## 4 ??+? 0.0128  0.0092 0.0104
## 5 ?+++ 0.1571  0.0033 0.0030
## 6 ?-?+ 0.4299  0.0005 0.0053
```

Notice that the sample size values in the column "Nsum" can vary from 9,703 to 444,006.

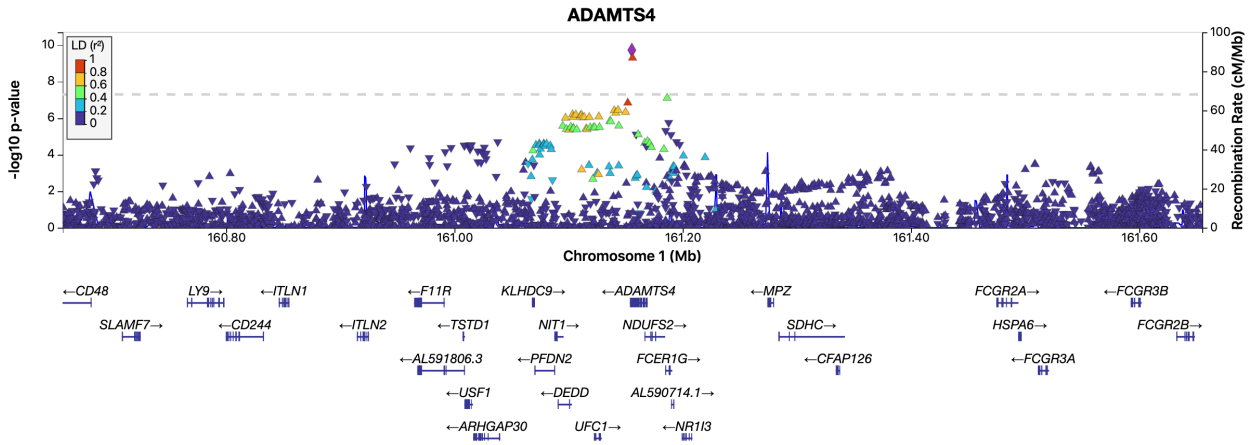


Figure 2: LocusZoom plot for ADAMTS4.

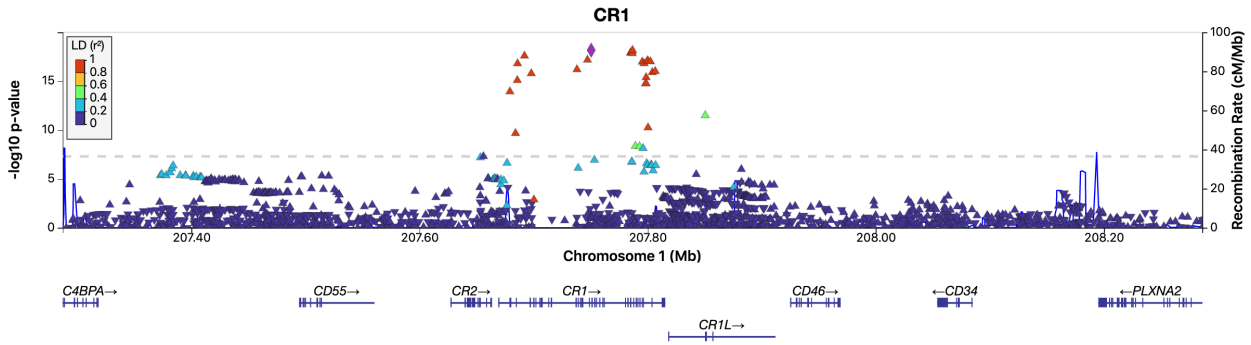


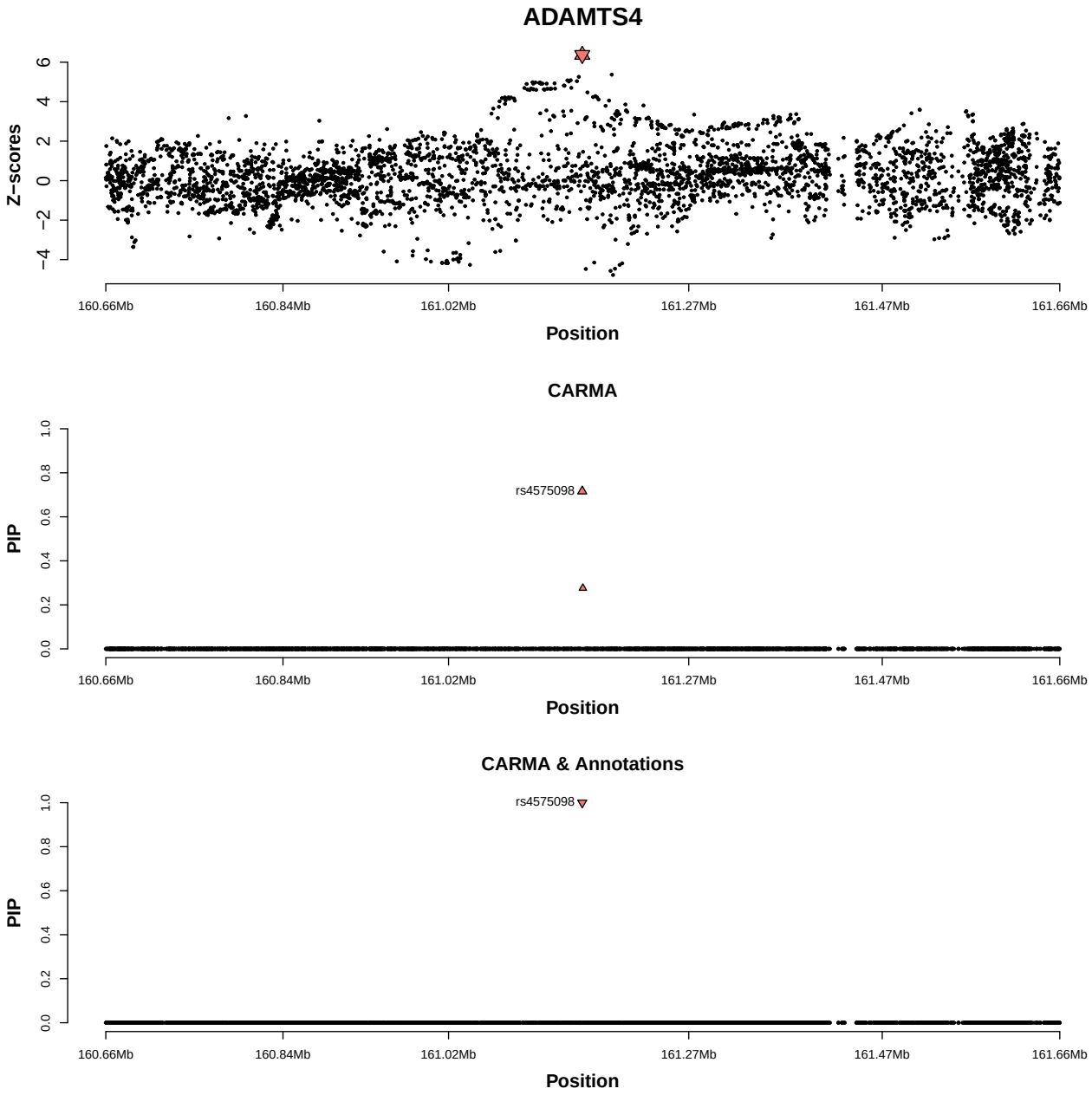
Figure 3: LocusZoom plot for CR1.

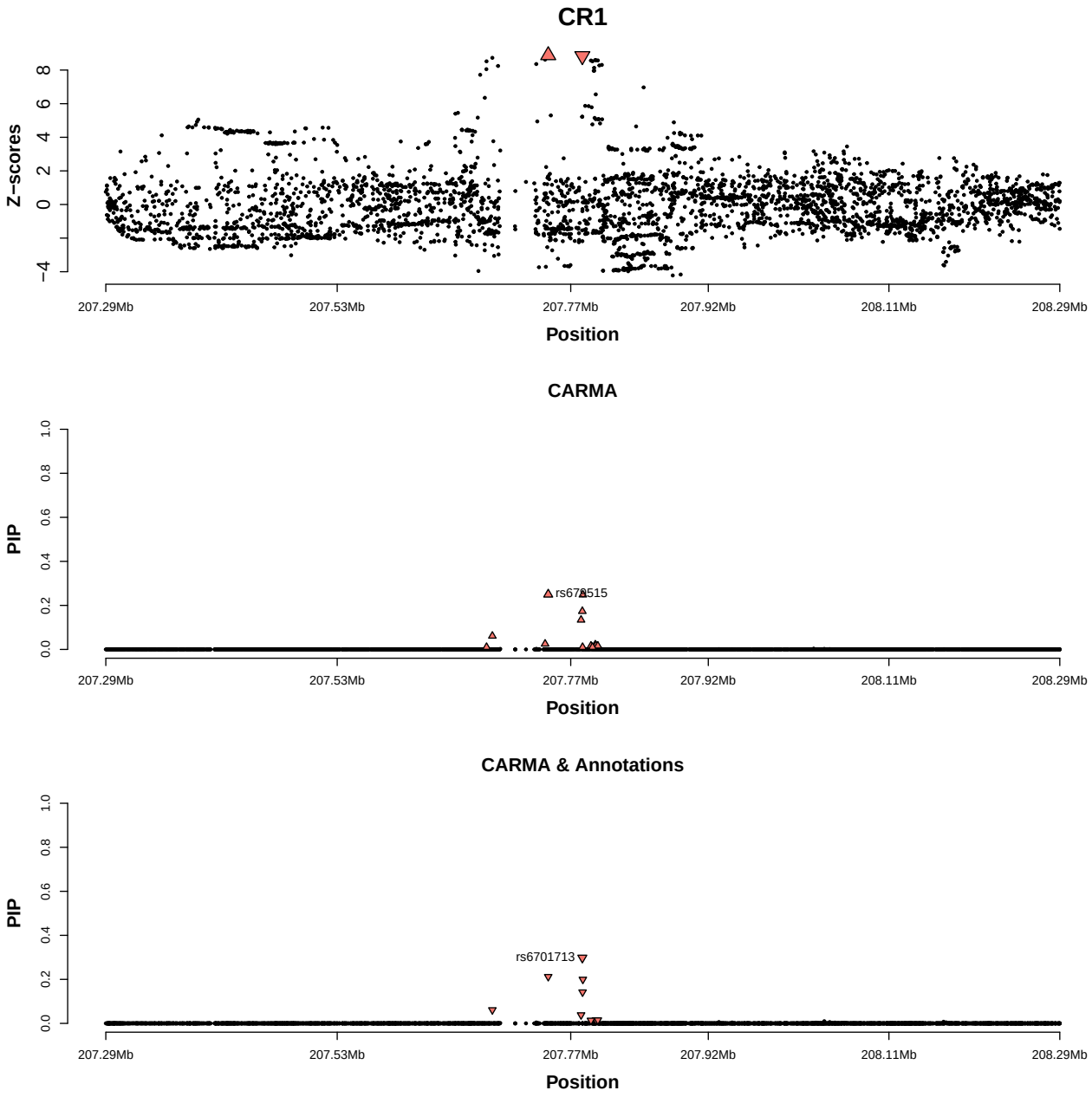
Next we run CARMA with two settings: 1. without annotations, and 2. with annotations as described above. We use the function "CARMA_fixed_sigma" to run CARMA with the external LD and $\eta = 1$ as the default setting. Given by that the LD matrix is extracted from reference panel (UKBB) instead of in-sample LD, we turn on the outlier detection setting 'outlier.switch=TRUE'.

```
###### load the GWAS summary statistics (part of AD GWAS sumstats from Jansen et al., 2019)
sumstat.1 =  fread(file = "Sample_data/ADAMTS4_sumstats.txt.gz",
                   sep = "\t", header = T, check.names = F, data.table = F,
                   stringsAsFactors = F)
sumstat.2 =  fread(file = "Sample_data/CR1_sumstats.txt.gz",
                   sep = "\t", header = T, check.names = F, data.table = F,
                   stringsAsFactors = F)

###### load the functional annotations for the variants included in
###### GWAS summary statistics (assuming the variants are sorted in
###### the same order as the variants in sumstat file)
annot.1 =  fread(file = "Sample_data/ADAMTS4_annotations.txt.gz",
                 sep = "\t", header = T, check.names = F, data.table = F,
                 stringsAsFactors = F)
annot.2 =  fread(file = "Sample_data/CR1_annotations.txt.gz",
                 sep = "\t", header = T, check.names = F, data.table = F,
                 stringsAsFactors = F)

###### load the pair-wise LD matrix (assuming the variants are sorted in
###### the same order as the variants in sumstat file)
ld.1 =  fread(file = "Sample_data/ADAMTS4_ld.txt.gz",
              sep = "\t", header = F, check.names = F, data.table = F,
              stringsAsFactors = F)
ld.2 =  fread(file = "Sample_data/CR1_ld.txt.gz",
              sep = "\t", header = F, check.names = F, data.table = F,
              stringsAsFactors = F)

z.list<-list()
ld.list<-list()
lambda.list<-list()
z.list[[1]]<-sumstat.1$Z
z.list[[2]]<-sumstat.2$Z
ld.list[[1]]<-as.matrix(ld.1)
ld.list[[2]]<-as.matrix(ld.2)
lambda.list[[1]]<-1
lambda.list[[2]]<-1
###### Without annotations
CARMA.results_no_annot<-CARMA_fixed_sigma(z.list,ld.list,lambda.list =  lambda.list,
                                          outlier.switch=T)

###### With annotations
###### Exclude the variant information columns in annotation file
###### such as positions and REF/ALT alleles.
annot.list<-list()
annot.list[[1]]<-as.matrix(cbind(1, annot.1 %>% select(-(uniqID.a1a2:SNP))))
annot.list[[2]]<-as.matrix(cbind(1, annot.2 %>% select(-(uniqID.a1a2:SNP))))

CARMA.results_annot<-CARMA_fixed_sigma(z.list,ld.list,w.list=annot.list,
                                       lambda.list =  lambda.list,
                                       input.alpha=0, outlier.switch=T)

###### Posterior inclusion probability (PIP) and credible set (CS)
sumstat.1 = sumstat.1 %>% mutate(PIP = CARMA.results_annot[[1]]$PIPs, CS = 0)
```

```
sumstat.1$CS[CARMA.results_annot[[1]]$`Credible set`[[2]][[1]]] = 1
sumstat.2 = sumstat.2 %>% mutate(PIP = CARMA.results_annot[[2]]$PIPs, CS = 0)
sumstat.2$CS[CARMA.results_annot[[2]]$`Credible set`[[2]][[1]]] = 1

###### write the GWAS summary statistics with PIP and CS
fwrite(x = sumstat.1, file = "Sample_data/ADAMTS4_carma.txt.gz",
       sep = "\t", quote = F, na = "NA", row.names = F, col.names = T, compress = "gzip")
fwrite(x = sumstat.2, file = "Sample_data/CR1_carma.txt.gz",
       sep = "\t", quote = F, na = "NA", row.names = F, col.names = T, compress = "gzip")
```

First, we examine the PIPs estimated by CARMA.





In the figure above, the credible sets are highlighted by colored shapes. Next, we can examine the SNPs included in the credible sets. For simplicity we only show the credible sets when including functional annotations.

```
carma_annot[[1]]$`Credible set` #the first element of the list
                                #is the result of the first locus ADAMTS4
```

```
## [1] "This is the first credible set of the locus ADAMTS4"
##      CHR        BP A1 A2       SNP   Z PIPs
## 2184   1 161155392  A  G rs4575098 6.4    1
```

```
carma_annot[[2]]$`Credible set` #the second element of the list
                                #is the result of the second locus CR1
```

```
## [1] "This is the first credible set of the locus CR1"
```

```
##      CHR        BP A1 A2       SNP   Z  PIPs
## 1563   1 207786289  A  G rs6701713 8.8 0.298
## 1426   1 207750568  T  C  rs679515 8.9 0.212
## 1570   1 207786828  A  G rs2093760 8.9 0.200
## 1567   1 207786542  A  G rs2093761 8.8 0.142
## 1343   1 207692049  A  G rs6656401 8.7 0.061
## 1559   1 207784968  A  G rs3818361 8.8 0.038
## 1626   1 207800555  T  C rs1408078 8.6 0.016
## 1629   1 207802552  A  C rs4844610 8.6 0.015
## 1598   1 207795320  A  G rs2296160 8.6 0.014
```

We can also examine the credible models.

```
carma_annot[[1]]$`Credible model`#the credible model of locus ADAMTS4
```

```
## [1] "This is the credible model of the locus ADAMTS4"
```

```
##      CHR        BP A1 A2       SNP   Z PIPs
## 2184   1 161155392  A  G rs4575098 6.4    1
```

```
carma_annot[[2]]$`Credible model`#the credible model of locus CR1
```

```
## [1] "This is the credible model of the locus CR1"
```

```
##      CHR        BP A1 A2       SNP   Z  PIPs
## 1563   1 207786289  A  G rs6701713 8.8 0.298
## 1426   1 207750568  T  C  rs679515 8.9 0.212
## 1570   1 207786828  A  G rs2093760 8.9 0.200
## 1567   1 207786542  A  G rs2093761 8.8 0.142
## 1343   1 207692049  A  G rs6656401 8.7 0.061
```

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