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 typical fine-mapping studies with two types of datasets:

- Summary statistics based on individual level phenotype and genotype data, and in-sample linkage disequilibrium (LD) matrix.
- Summary statistics generated by meta-analysis, and LD matrix extracted from reference panels.

Environment preparation

Install the package 'CARMA'.

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

Create a folder 'CARMA' (or any other folder that will store the demo data), 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
```

Check if the following packages are installed, which will be used in this tutorial.

```
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")
}
```

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 a Gaussian regression model with the simulated

genotypes $\boldsymbol{X}$:

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

where $\boldsymbol{\beta}$ 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. The probability of a variant being causal is computed based on 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 of locus chr1: 200,937,832-201,937,832

In this section, we use the simulated data based on the locus chr1:200937832-201937832. We computed the summary statistics (Z-scores) and the LD matrix. The pre-determined causal SNPs are the **287**th, **1275**th, and **2572**th SNPs at the locus (the left, middle, right red points respectively).
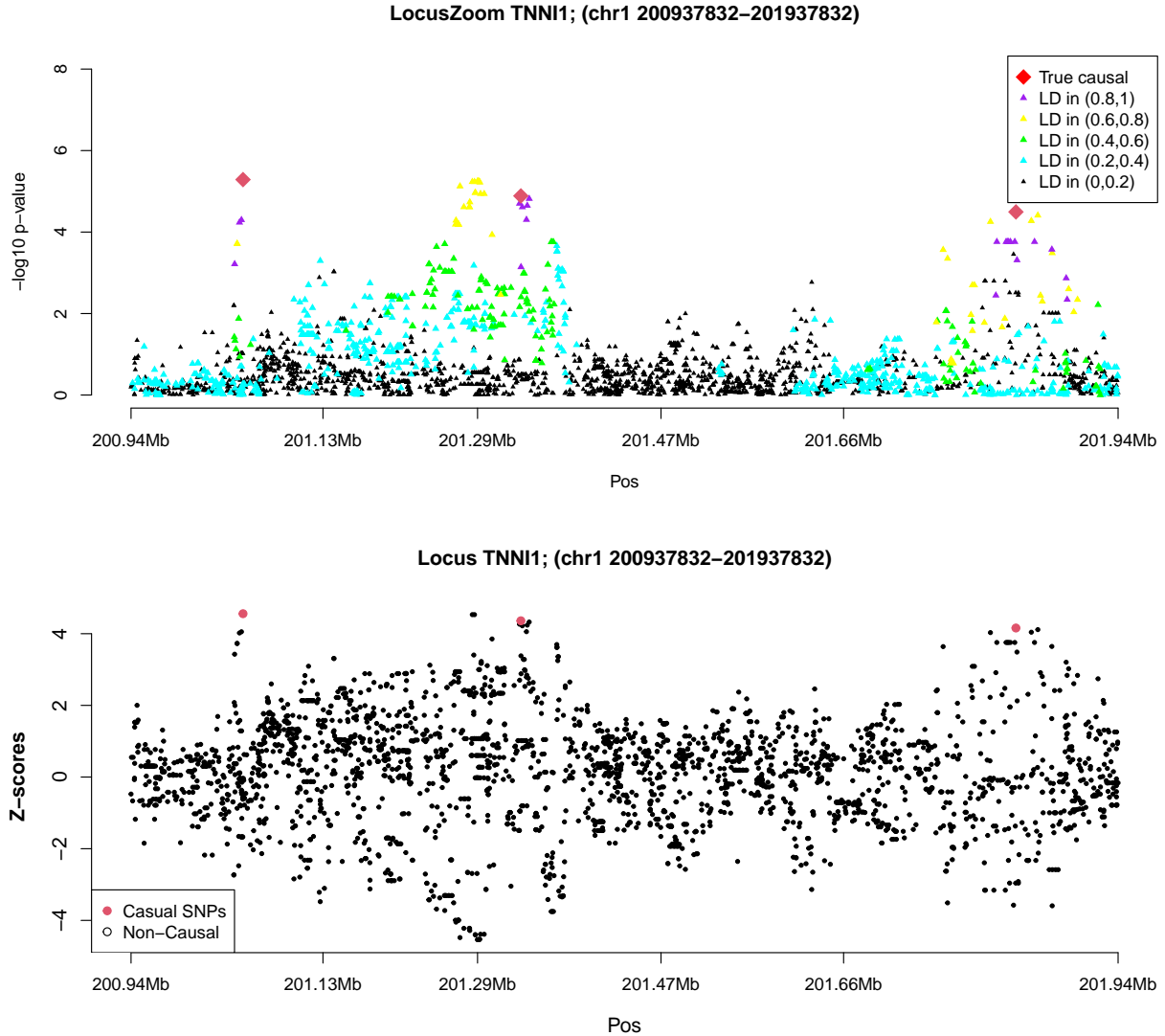

Figure 1: LocusZoom and Z-score plots for locus chr1:200,937,832-201,937,832

As shown in the figure below, one of the causal SNP has few highly correlated SNPs and larger Z-scores, whereas the other two SNPs are highly correlated to the surrounding SNPs with similar values of 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'' function in the package, which is designed to run in-sample data. As recommended in the paper, we choose the dimensional hyperparameter η as $1/\sqrt{p}$, where p is the total number of SNPs at the 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.

```r
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 of GWAS with 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
```
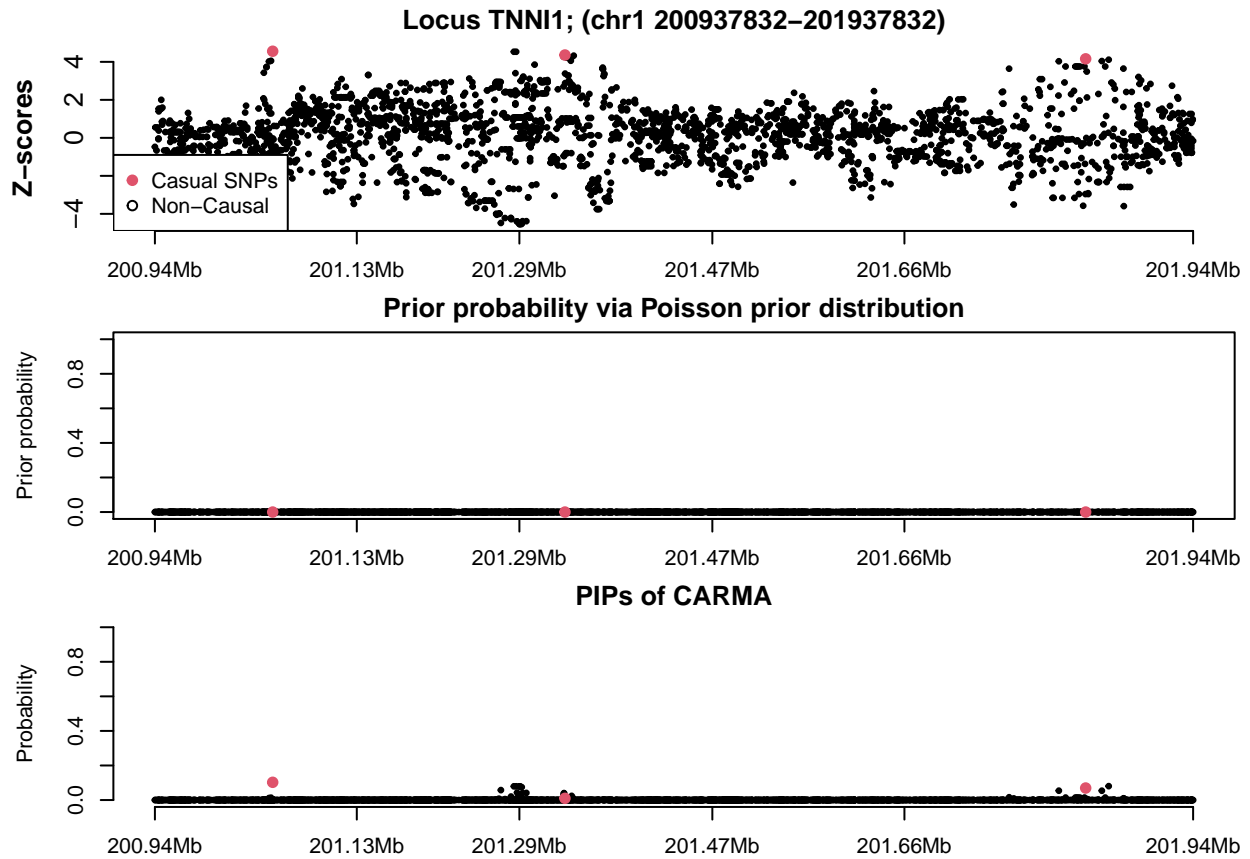
Next, we run CARMA with the input summary statistics Z and the LD matrix with the hyperparameter $eta = 1$ as the default setting. Notice that given the fact that the LD matrix is in-sample, we turn off the outlier detection by the setting 'outlier.switch=F'.

```r
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.

Locus TNNI1; (chr1 200937832–201937832)

Prior probability via Poisson prior distribution

PIPs of CARMA


```
##      SNPs.index Causal.status Z.scores  PIPs
## 287         287   True causal      4.6 0.103
## 2603       2603    Non-causal      4.1 0.080
## 1095       1095    Non-causal      4.5 0.079
## 1105       1105    Non-causal      4.5 0.079
## 1120       1120    Non-causal     -4.5 0.079
## 1121       1121    Non-causal     -4.5 0.079
## 1125       1125    Non-causal     -4.5 0.079
## 1134       1134    Non-causal     -4.5 0.075
## 2572       2572   True causal      4.2 0.069
## 1057       1057    Non-causal     -4.5 0.057
## 2530       2530    Non-causal      4.0 0.054
## 2591       2591    Non-causal      4.0 0.054
## 1110       1110    Non-causal     -4.4 0.043
## 1141       1141    Non-causal     -4.4 0.041
## 1158       1158    Non-causal     -4.4 0.041
## 1272       1272    Non-causal      4.4 0.040
## 1273       1273    Non-causal      4.4 0.040
## 1268       1268    Non-causal      4.3 0.027
## 1298       1298    Non-causal      4.2 0.024
```

```
## 1131        1131    Non-causal     -4.5 0.021
```

We can observe that the 287th SNP (the causal SNP at left), which is a true causal SNP with a larger Z-score comparing to other highly correlated SNPs, received a medium PIP value comparing to the surrounding SNPs. On the other hand, the other two causal SNPs, which are highly correlated to other 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 1134 1057 1109 1110 1141 1158 1272 1273
## [16] 1275 1304 2603 1086 1268 2572 1298 1063 1082 1084 1281 2530 2591 1294  269
## [31] 1043  257 1041 1048 1050 1051 1053
```

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. Notice that the PIPs shown above might not be able to be exactly replicated due to the setting of different seeds, however, the conclusion is the same as there are two causal variants that are indistinguishable to the other highly correlated non-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.

Locus TNNI1; (chr1 200937832–201937832)

Prior probability via Poisson prior distribution

PIPs of CARMA
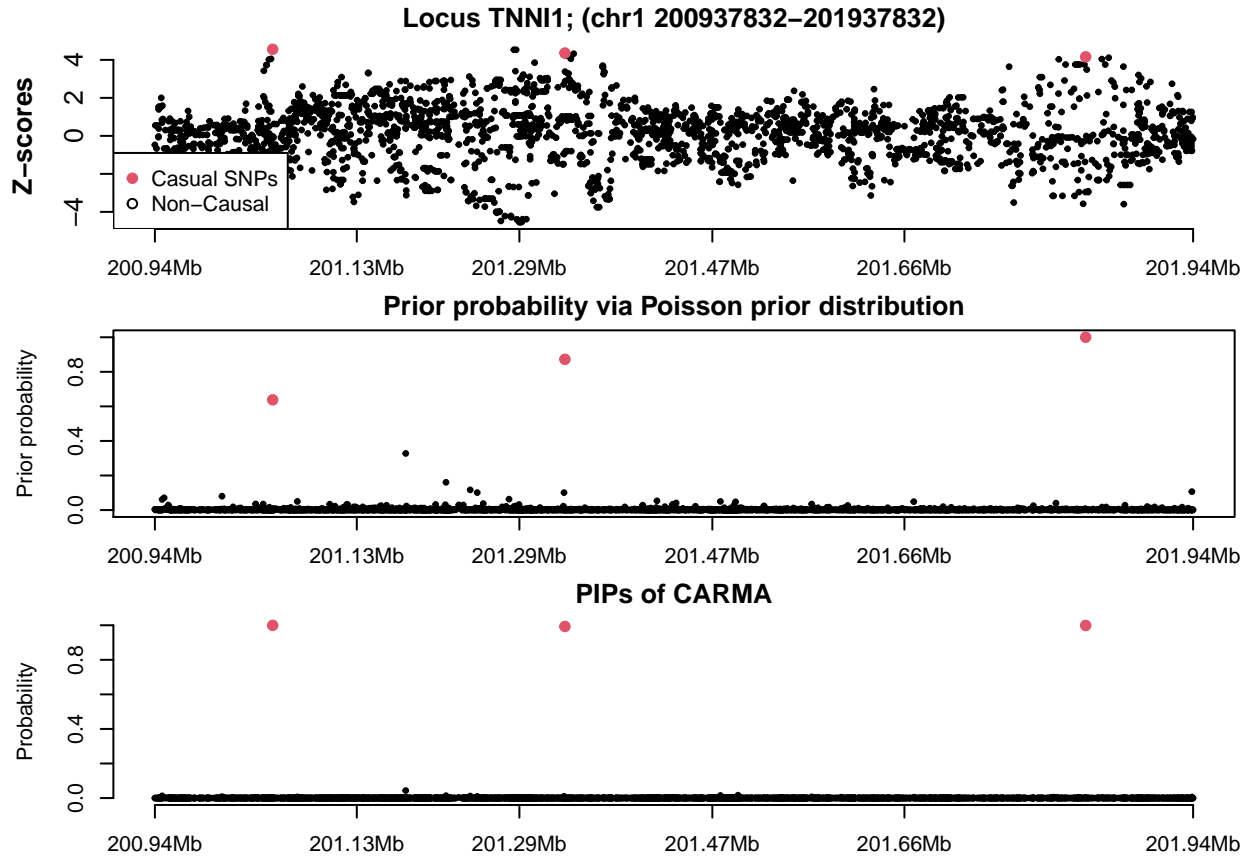
```
##      SNPs.index Causal.status Z.scores   PIPs
## 287         287   True causal     4.56 0.9996
## 2572       2572   True causal     4.16 0.9988
## 1275       1275   True causal     4.36 0.9930
## 724         724    Non-causal     0.38 0.0436
## 1827       1827    Non-causal    -2.58 0.0166
## 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 numbers of included SNPs in credible models, which only include to the three true causal SNPs, have been reduced significantly. Also, the credible sets strengthened by the annotations identified the three true causals.

Notice that, the results can be different from the results shown in the tutorial due to different seeds being used. Also, notice that the result of CARMA based on annotations shown in here is the result of the simulation study in the main manuscript, which is based on 9 loci in chromosome 1. In here, we only demonstrate CARMA with one locus for simplicity.

Summary statistics and LD matrix extracted from reference panels

Usually, individual level data are not available in large 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 the 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
```

From the AD data we use Z-scores. Notice that the sample size values in the column "Nsum" can vary from 9,703 to 444,006 depending on which datasets are included in the meta-analyses of the AD study.
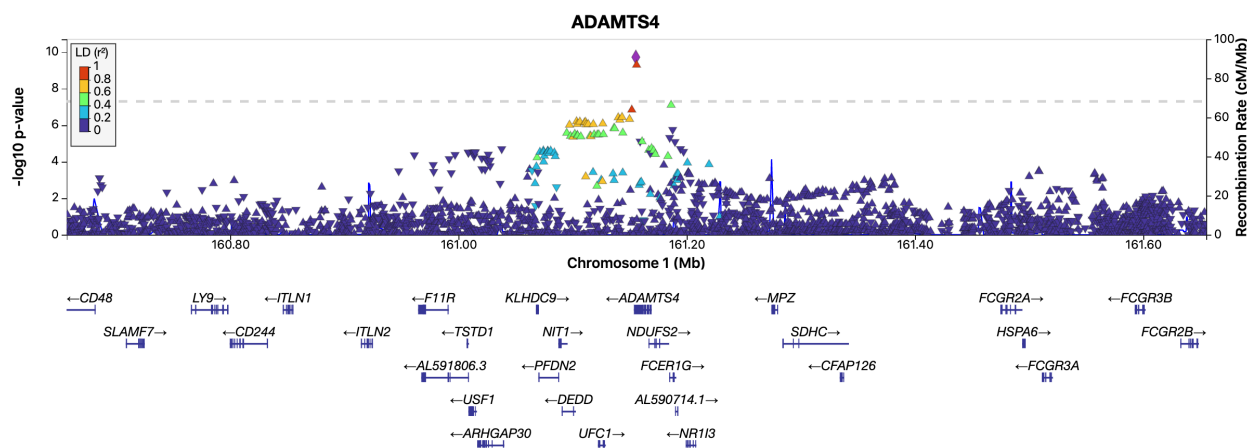

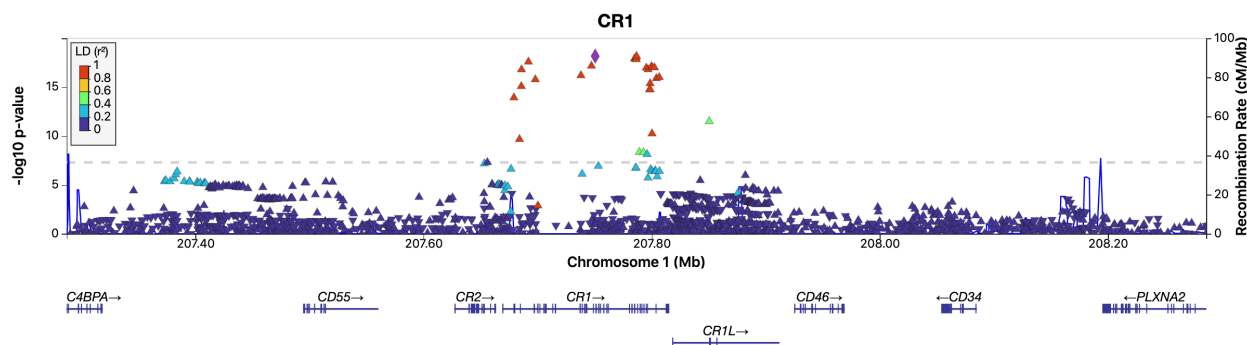Figure 2: LocusZoom plot for ADAMTS4.

Figure 3: LocusZoom plot for CR1.

Next we run CARMA with two settings: 1. without annotations, and 2. with annotations as described above.

We still use the function "CARMA_fixed_sigma" to run the results of meta-analysis with the external LD and $\eta = 1$ as the default setting. Given the fact that the LD matrix is extraced from reference panel (UKBB) instead of in-sample LD, we turn on the outlier detection as 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.
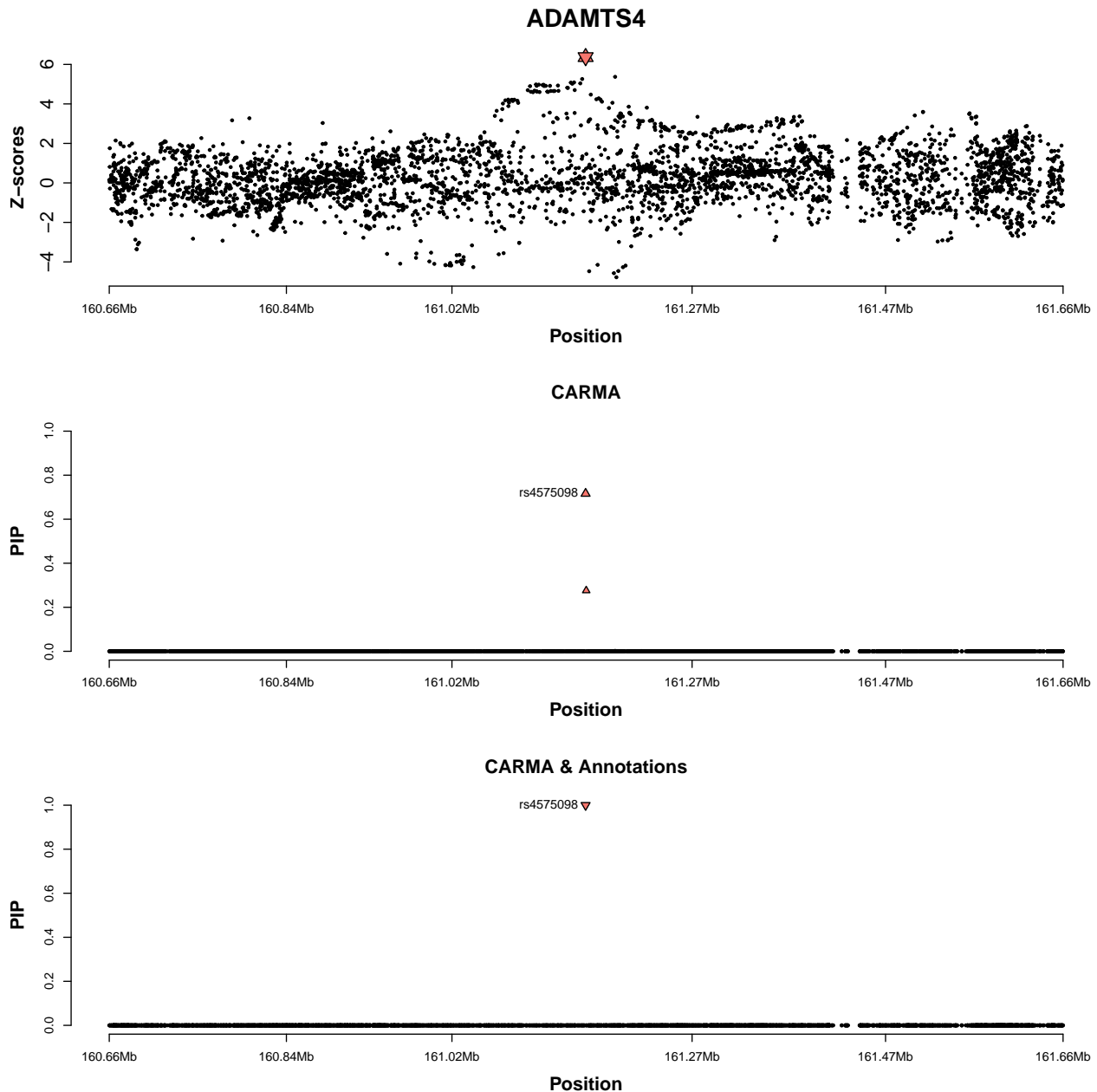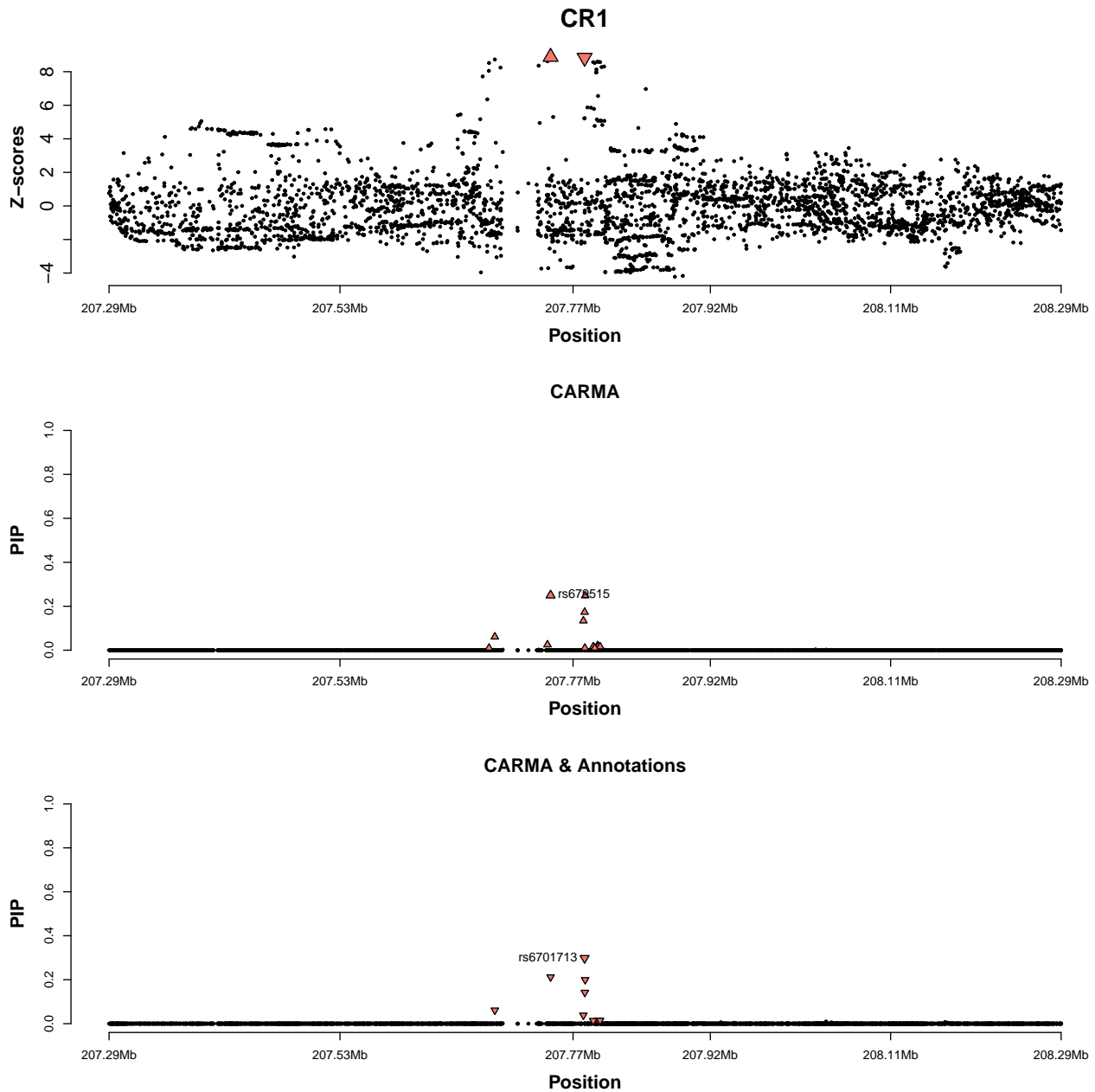
In the figure above, the credible sets are highlighted by colored shapes. Next, we can examine the SNPs included in the credible sets. For simplicity we only show the credible sets when including functional annotations.

```
## [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

## [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.

```
## [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
```

```
## [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
```

References

Dimitromanolakis, Apostolos, Jingxiong Xu, Agnieszka Krol, and Laurent Briollais. 2019. "sim1000G: A User-Friendly Genetic Variant Simulator in r for Unrelated Individuals and Family-Based Designs." *BMC Bioinformatics* 20 (1): 26.

Jansen, Iris E, Jeanne E Savage, Kyoko Watanabe, Julien Bryois, Dylan M Williams, Stacy Steinberg, Julia Sealock, et al. 2019. "Genome-Wide Meta-Analysis Identifies New Loci and Functional Pathways Influencing Alzheimer's Disease Risk." *Nature Genetics* 51 (3): 404–13.

Weissbrod, Omer, Farhad Hormozdiari, Christian Benner, Ran Cui, Jacob Ulirsch, Steven Gazal, Armin P Schoech, et al. 2020. "Functionally Informed Fine-Mapping and Polygenic Localization of Complex Trait Heritability." *Nature Genetics*, 1–9.