CARMA Tutorial

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4/21/2022

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 being causal. 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.

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

Also, open terminal and download the example datasets from GitHub repository ZikunY/CARMA. The sample data is downloaded from the GitHub repo, and the file path of demo data should be under the repo folder of the git clone unless the user setwd to the git clone directory.

```
git clone https://github.com/ZikunY/CARMA.git
```

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 $\boldsymbol{X}$:

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

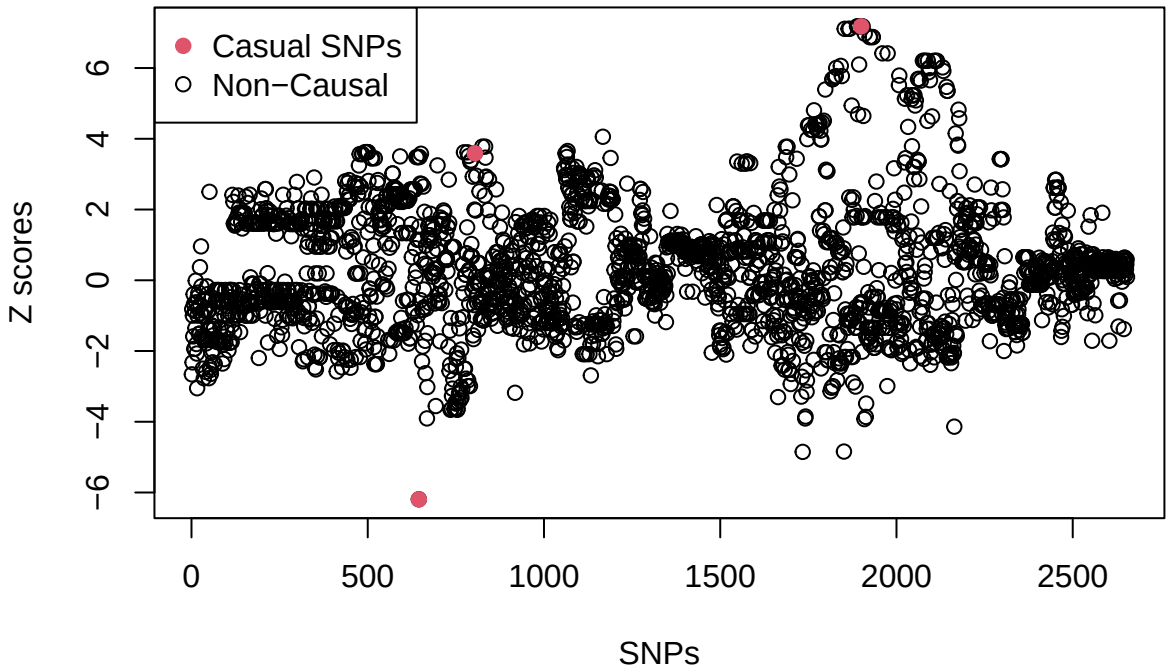
where $\boldsymbol{\beta}$ is a sparse coefficient vector such as $\beta_i \neq 0$ if the ith SNP is a causal SNP, and $\boldsymbol{\epsilon}$ is the standard Gaussian error. The probability of a variant being causal is decided through a logistic regression model such that

$$\Pr\{\beta_i \neq 0 | \boldsymbol{w}_i, \boldsymbol{\theta}\} = \frac{1}{1+\exp\{-\boldsymbol{w}_i'\boldsymbol{\theta}\}},$$

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 128952507-129961171 on chromosome 11

In this section, we use the simulated data based on the locus chr11:128952507-129961171. We computed the summary statistics (Z-scores) and the LD matrix. The pre-determined causal SNPs are the **645**th, **804**th, and **1900**th SNPs at the locus.

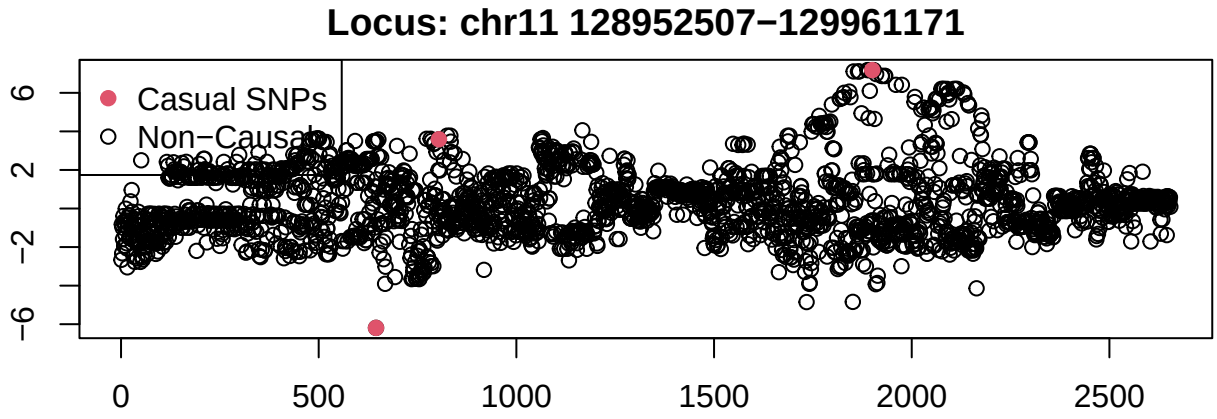


As shown in the figure, one of the causal SNP is relatively independent of the other SNPs, whereas the other two SNPs are highly correlated to the surrounding SNPs with similar values of Z-scores.

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.

```
setwd('CARMA/Sample_data') ### setting up the working directory or the wd where the data are stored
data<-readRDS('in-sample_data.RData')
z.list<-list()
ld.list<-list()
lambda.list<-list()
z.list[[1]]<-data$Z
ld.list[[1]]<-data$LD
lambda.list[[1]]<-1/sqrt(nrow(ld.list[[1]]))
CARMA.results<-CARMA(z.list,ld.list,lambda.list=lambda.list)
```

We can check the results.




```
##       SNPs.index Causal.status  Z.scores        PIPs
## 645          645   True causal -6.191607 0.999998111
## 1888        1888    Non-causal  7.178537 0.181027464
## 1892        1892    Non-causal  7.178537 0.181027464
## 1900        1900   True causal  7.178537 0.181027464
## 1904        1904    Non-causal  7.178537 0.181027464
## 1853        1853    Non-causal  7.108896 0.074692442
## 1867        1867    Non-causal  7.108896 0.074692442
## 1864        1864    Non-causal  7.108896 0.069958505
## 1910        1910    Non-causal  6.972467 0.022387332
## 825          825    Non-causal  3.778803 0.011750509
## 833          833    Non-causal  3.778803 0.011750509
## 1924        1924    Non-causal  6.874909 0.008507082
## 1925        1925    Non-causal  6.874909 0.008507082
## 1932        1932    Non-causal  6.874909 0.008507082
## 1933        1933    Non-causal  6.874909 0.008507082
## 773          773    Non-causal  3.616948 0.005337156
## 783          783    Non-causal  3.616948 0.005337156
## 804          804   True causal  3.590660 0.004749275
## 786          786    Non-causal  3.513737 0.002994474
## 834          834    Non-causal  3.465383 0.001743837
```

We can observe that the 645th SNP, which is a true causal SNP and fairly independent of other SNPs, received a large PIP value. On the other hand, the 1900th SNP, which is also a true causal SNP and highly correlated to other surrounding SNPs, shared the PIP with the highly correlated SNPs. We can also check the credible sets and credible models.

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

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

```
## [1] 645
##
## [[2]]
##  [1] 1888 1892 1900 1904 1853 1867 1864 1910 1924 1925 1932
```

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

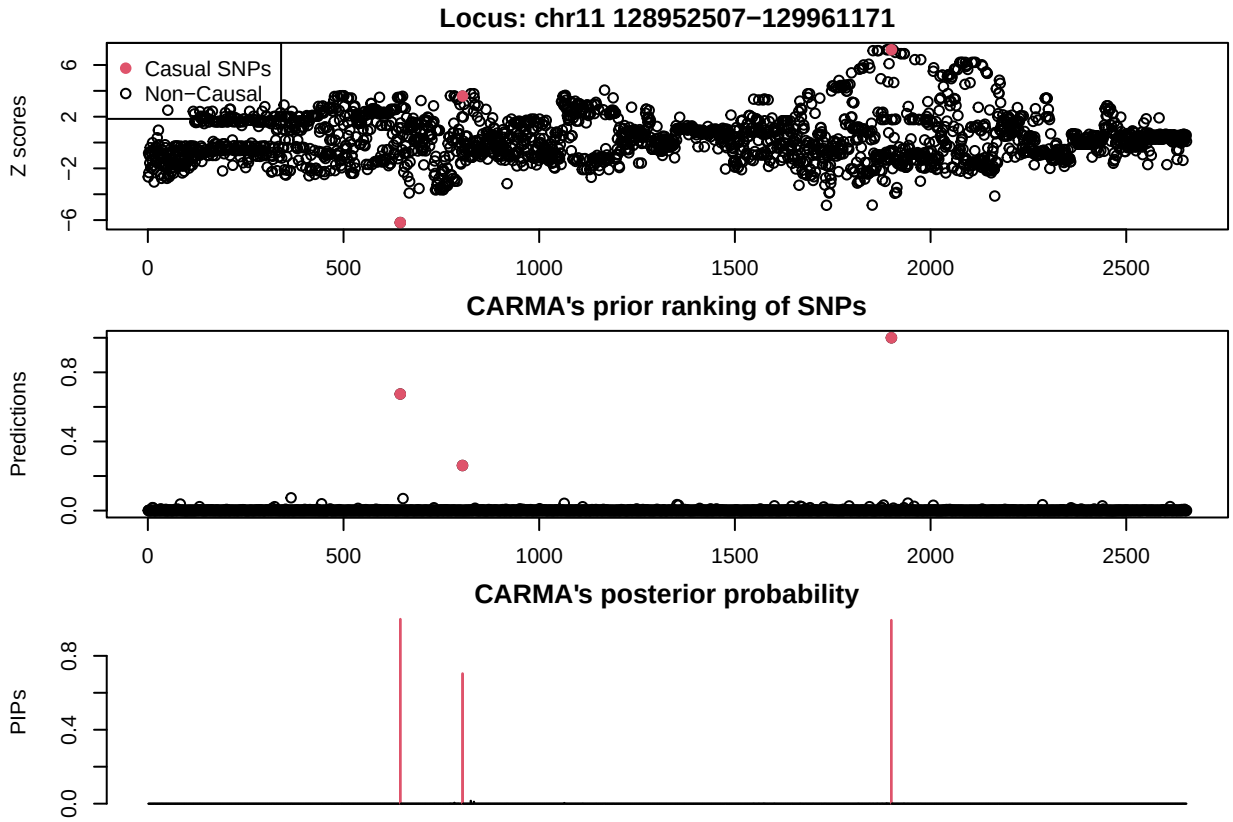
```
## [1]  645 1892 1888 1900 1904 1853 1864 1867 1910
```

Given the threshold for the credible set $\rho = 0.99$, the 645th SNP formulate a credible set with single SNP. The second credible set, which include the true causal SNP (the 1900th SNP), includes 11 SNPs with a minimum LD 0.894 among the SNPs. The number of SNPs identified by the credible model is 9, which is smaller than for the credible set.

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

```
data<-readRDS('in-sample_data.RData')
z.list<-list()
ld.list<-list()
lambda.list<-list()
annot.list<-list()
z.list[[1]]<-data$Z
ld.list[[1]]<-data$LD
lambda.list[[1]]<-1/sqrt(nrow(ld.list[[1]]))
annot.list[[1]]<-data$Annotations
CARMA.results<-CARMA(z.list,ld.list,lambda.list=lambda.list,w.list=annot.list)
```

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



```
##      SNPs.index Causal.status  Z.scores         PIPs
## 645         645   True causal -6.191607 0.9999999961
## 1900       1900   True causal  7.178537 0.9943640877
## 804         804   True causal  3.590660 0.7056123753
## 825         825    Non-causal  3.778803 0.0159377924
## 833         833    Non-causal  3.778803 0.0106795049
## 783         783    Non-causal  3.616948 0.0051777324
## 1064       1064    Non-causal  3.594629 0.0040586959
## 1888       1888    Non-causal  7.178537 0.0023769107
## 786         786    Non-causal  3.513737 0.0011411282
## 834         834    Non-causal  3.465383 0.0010816345
## 1574       1574    Non-causal  3.356648 0.0010565376
## 773         773    Non-causal  3.616948 0.0010400024
## 1932       1932    Non-causal  6.874909 0.0008463874
## 1548       1548    Non-causal  3.356648 0.0007822398
## 1877       1877    Non-causal -2.890220 0.0007598126
## 1111       1111    Non-causal  2.535731 0.0006876151
## 1892       1892    Non-causal  7.178537 0.0006706814
## 1601       1601    Non-causal  1.133419 0.0006540633
## 793         793    Non-causal  3.389752 0.0006520183
## 1904       1904    Non-causal  7.178537 0.0005534825
```

As observed from the figure above, the prior helps CARMA distinguish the true causal variants from the highly correlated SNPs, such as the 1900th SNP which in the absence of functional annotations cannot be distinguished from other highly correlated SNPs. Also, the 804th SNP which was missed before receives a high PIP this time. We can also examine the credible sets and credible models of CARMA.

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

```
## [[1]]
## [1] 645
##
## [[2]]
## [1] 1900
```

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

```
## [1]  645  804 1900
```

The numbers of included SNPs in both credible sets and credible models have been reduced significantly, with the top candidate model successfully identifying the three causal SNPs.

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 924 functional annotations including DeepSEA (Zhou and Troyanskaya 2015), CADD (Kircher et al. 2014), PO-EN (Yang et al. 2021), and PolyFun (Weissbrod et al. 2020).

Demonstration with the loci ADAMTS4 and CR1

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

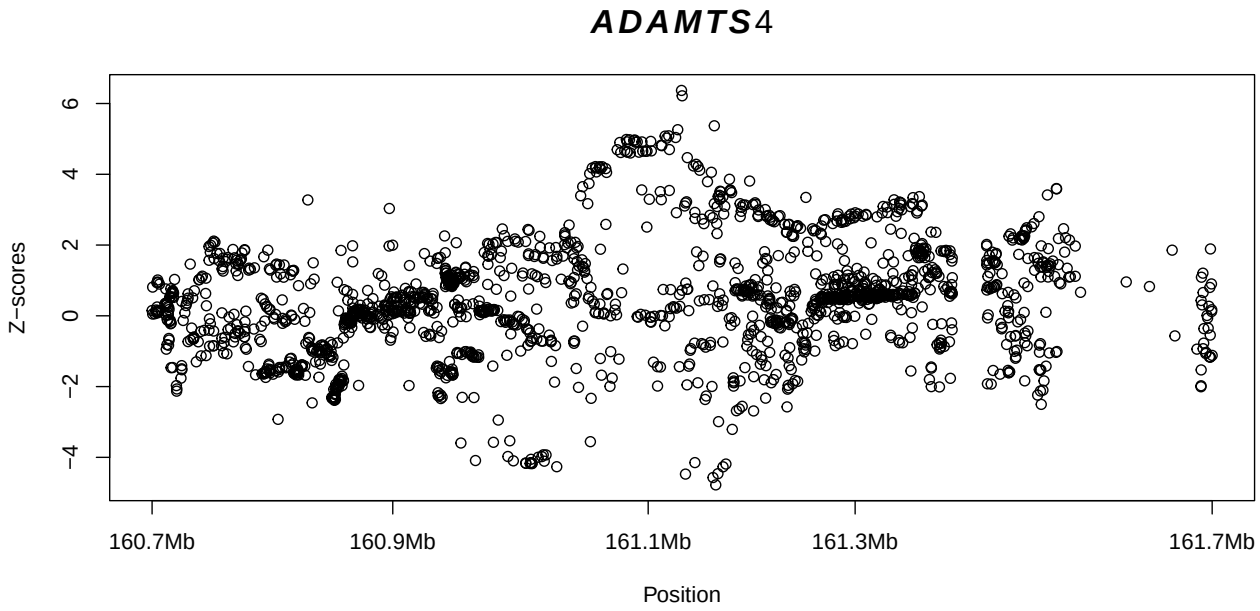
Data of the locus ADAMTS4

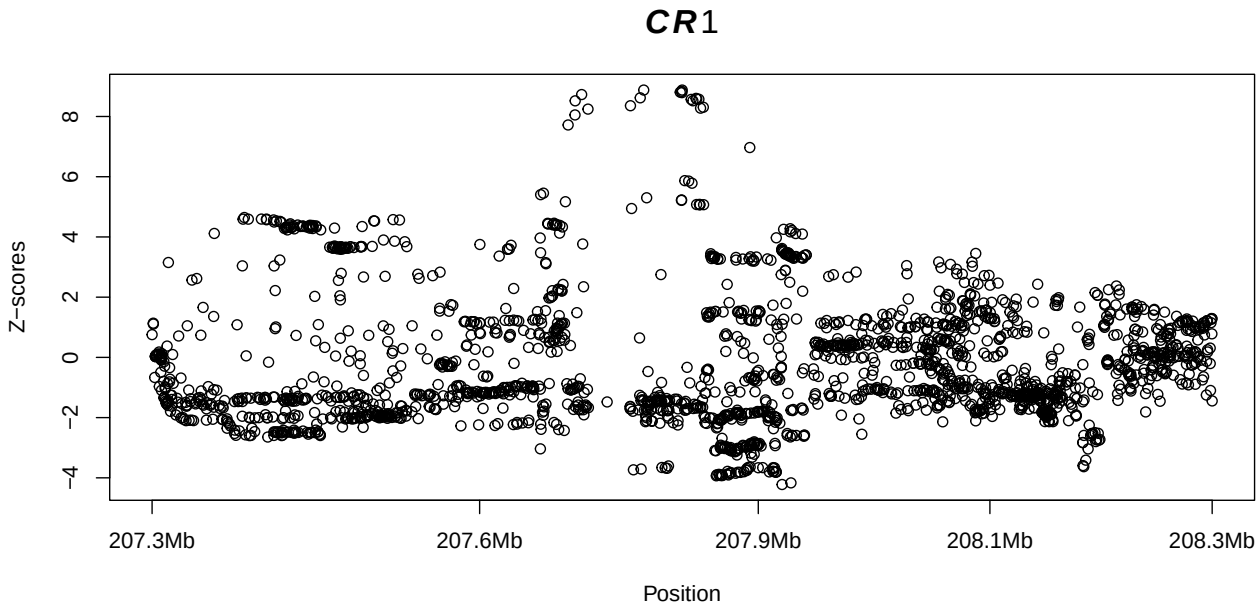
```
##       uniqID.a1a2 CHR        BP A1 A2         SNP          Z         P   Nsum
## 1 1:160656603_A_T   1 160656603  A  T   rs6702441 0.14921734 0.8813821 429975
## 2 1:160657127_T_C   1 160657127  T  C rs143426473 0.81166395 0.4169845 435185
## 3 1:160657137_G_A   1 160657137  G  A  rs11589131 0.04962457 0.9604216 429757
## 4 1:160659160_A_G   1 160659160  A  G  rs12117768 0.05811025 0.9536608 434113
## 5 1:160660312_C_T   1 160660312  C  T  rs11265464 0.16425553 0.8695300 436498
## 6 1:160660590_C_G   1 160660590  C  G  rs12143827 0.11044131 0.9120594 436064
##       Neff  dir       EAF         BETA          SE
## 1 423496.9 ?+-+ 0.3695570 0.0003359043 0.002251107
## 2 428659.9 ?+++ 0.0337234 0.0048561053 0.005982901
## 3 423280.9 ?+-+ 0.3693410 0.0001117523 0.002251954
## 4 427597.5 ?+-+ 0.3694330 0.0001301927 0.002240443
## 5 429961.1 ?+-+ 0.3689250 0.0003670970 0.002234914
## 6 429531.0 ?+-+ 0.3714350 0.0002466055 0.002232910
```

Data of the locus CR1

```
##       uniqID.a1a2 CHR        BP A1 A2        SNP           Z         P   Nsum
## 1 1:207287187_T_C   1 207287187  T  C  rs2808470  0.75499216 0.4502537 433909
## 2 1:207288297_T_C   1 207288297  T  C rs17020983  1.13570436 0.2560803 434723
## 3 1:207288392_G_A   1 207288392  G  A rs17020993  1.10788037 0.2679135 436498
## 4 1:207289643_T_G   1 207289643  T  G rs61525336 -0.66705148 0.5047392 431994
## 5 1:207290247_T_C   1 207290247  T  C  rs4844568  0.04109237 0.9672223 434335
## 6 1:207290328_T_C   1 207290328  T  C  rs4844569  0.02436483 0.9805616 434360
##       Neff  dir       EAF          BETA          SE
## 1 427395.4 ?-++ 0.1939640  2.065259e-03 0.002735470
## 2 428202.0 ?-++ 0.0960117  4.165641e-03 0.003667892
## 3 429961.1 ?+++ 0.1570680  3.283391e-03 0.002963669
## 4 425497.7 ?+-- 0.1227290 -2.203713e-03 0.003303663
## 5 427817.5 ?++- 0.3598860  9.255626e-05 0.002252395
## 6 427842.3 ?+-- 0.3599480  5.487557e-05 0.002252245
```

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.



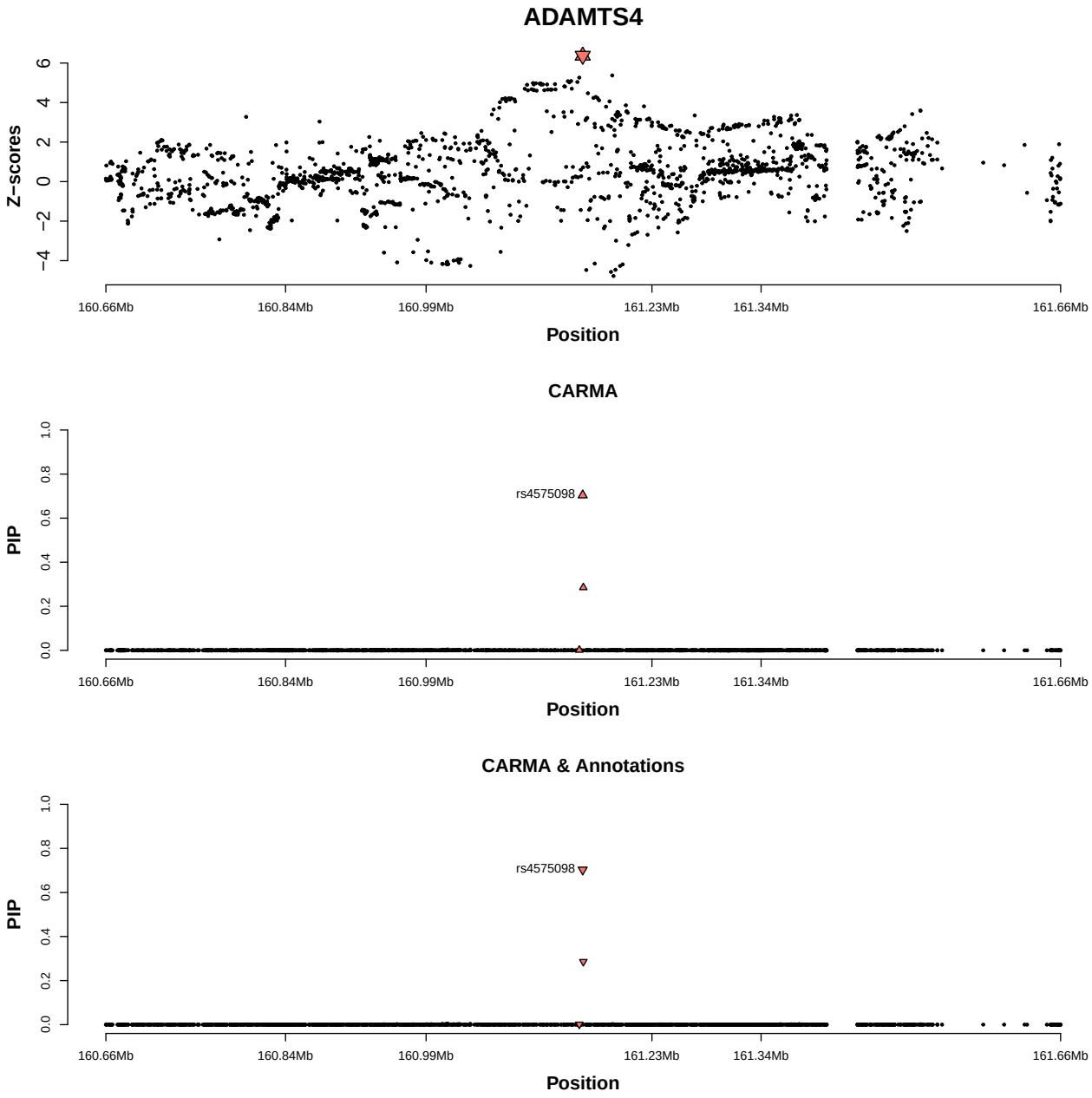


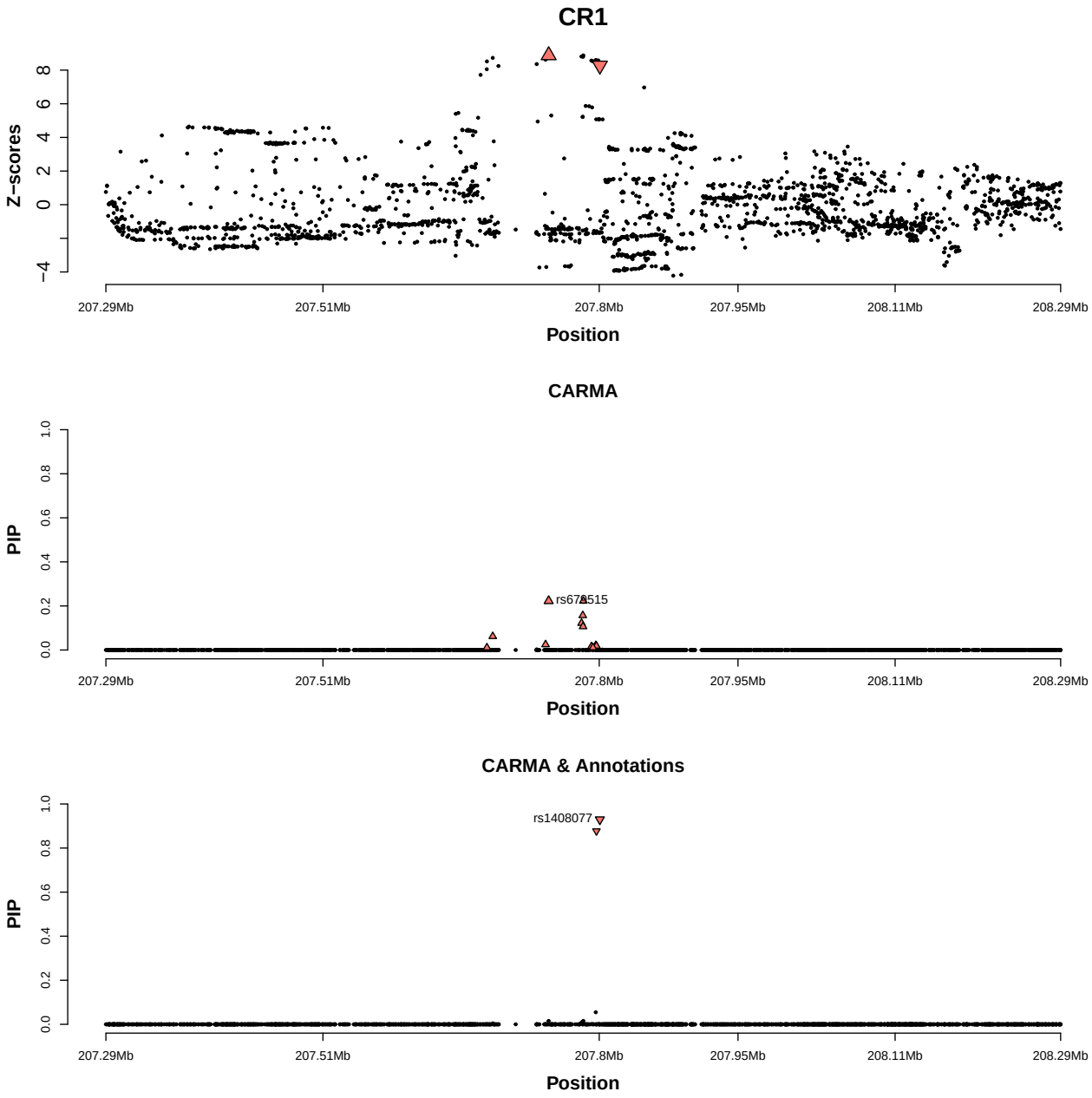
Next we run CARMA with two settings: 1. without annotations, and 2. with annotations as described above. We use the function "CARMA_fixed_sigma" to run the meta-analysis and the external LD. Also, the hyperparameter η is chosen by an adaptive procedure to control the false positives caused by possible discrepancies between Z-scores and external LD values.

```
Data_ADAMTS4<-readRDS('ADAMTS4.RData')
Data_CR1<-readRDS('CR1.RData')
z.list<-list()
ld.list<-list()
z.list[[1]]<-Data_ADAMTS4$`Meta-data`$Z
z.list[[2]]<-Data_CR1$`Meta-data`$Z
ld.list[[1]]<-Data_ADAMTS4$LD
ld.list[[2]]<-Data_CR1$LD
CARMA.results_no_annot<-CARMA_fixed_sigma(z.list,ld.list)
```

```
######With annotations
######The first 6 column of annotation file include location information etc.
annot.list<-list()
annot.list[[1]]<-as.matrix(cbind(1,Data_ADAMTS4$Annotations[,-(1:6)])
annot.list[[2]]<-as.matrix(cbind(1,Data_CR1$Annotations[,-(1:6)])
CARMA.results_annot<-CARMA_fixed_sigma(z.list,ld.list,w.list=annot.list)
```

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
## 991   1 161155392  A  G  rs4575098 6.369505 0.703105969
## 993   1 161156033  A  C rs11585858 6.217633 0.285750659
## 987   1 161151844  A  G rs11265563 5.260424 0.001367149
```

```
## [1] "This is the first credible set of the locus CR1"
##     CHR        BP A1 A2       SNP        Z      PIPs
## 894   1 207804141  A  C rs1408077 8.276385 0.9294497
## 885   1 207800555  T  C rs1408078 8.582403 0.8773540
```

We can also examine the credible models.

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

```
##     CHR        BP A1 A2        SNP        Z      PIPs
## 991   1 161155392  A  G  rs4575098 6.369505 0.7031060
## 993   1 161156033  A  C rs11585858 6.217633 0.2857507
```

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

```
##     CHR        BP A1 A2       SNP        Z      PIPs
## 885   1 207800555  T  C rs1408078 8.582403 0.8773540
## 894   1 207804141  A  C rs1408077 8.276385 0.9294497
```

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.

Kircher, Martin, Daniela M Witten, Preti Jain, Brian J O'roak, Gregory M Cooper, and Jay Shendure. 2014. "A General Framework for Estimating the Relative Pathogenicity of Human Genetic Variants." *Nature Genetics* 46 (3): 310–15.

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.

Yang, Zikun, Chen Wang, Stephanie Erjavec, Lynn Petukhova, Angela Christiano, and Iuliana Ionita-Laza. 2021. "A Semisupervised Model to Predict Regulatory Effects of Genetic Variants at Single Nucleotide Resolution Using Massively Parallel Reporter Assays." *Bioinformatics* 37 (14): 1953–62.

Zhou, Jian, and Olga G Troyanskaya. 2015. "Predicting Effects of Noncoding Variants with Deep Learning–Based Sequence Model." *Nature Methods* 12 (10): 931–34.