Using RQT, an R/Bioconductor package for gene-level meta-analysis

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1 Overview

Despite the recent advances of modern GWAS methods, it is still remains an important problem of addressing calculation an effect size and corresponding p-value for the whole gene rather than for single variant. We developed an R-package rqt, which offers gene-level GWAS meta-analysis. The package can be easily included into bioinformatics pipeline or used as stand-alone. Contact: ilya.zhbannikov@duke.edu for questions of usage the rqt or any other issues.

Below we provide several examples that show GWAS meta-analysis on gene-level layer.

1.1 Methods in brief

The workflow of gene-level meta analysis consists of the following steps: (i) reducing the number of predictors, thereby alleviating correlation problem in variants (accounting for LD); (ii) then the regression mod-el is fitted on the reduced dataset to obtain corresponding regression coefficient ("effect sizes"); (iii) these coefficients are then to be pooled into a total index representing a total gene-level effect size and corresponding statistics is calculated. P- and q- values are then calculated using this statistics from asymptotic approximation or permutation procedure; (iv) the final step is combining gene-level p-values calculated from each study with Fisher's combined probability method.

2 Installation

2.1 Most-recent version from GitHub

> require(devtools)

> devtools::install_github("izhbannikov/rqt")

3 Data description

3.1 Single dataset

In rqt requires the following datasets: (i) phenotype (a N by 1) matrix (i.e. a vector); and (ii) genotype - an object of class SummarizedExperiment containing one assay: (a N by M) matrix, where N - is the total number of individuals in the study and M is the total number of genetic variants. Optionally, rqt can accept covariates, in form of N by K matrix, where K is the total number of covariates used in the study. Phenotype can be dichotomous (0/1, where 1 indicates control and 0 case).

3.2 Meta-analysis

In meta-analysis, rqt requires a list of M (M - number of datasets used in meta-analysis) and optionally it accepts covariates in form described above.

4 Examples

4.1 Gene-level analysis on a single dataset

4.2 Dichotomous phenotype

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> library(rgt)
> data <- data.matrix(read.table(system.file("extdata/test.bin1.dat",</pre>
                                                   package="rqt"), header=TRUE))
> pheno <- data[,1]</pre>
> geno <- data[, 2:dim(data)[2]]
> colnames(geno) <- paste(seq(1, dim(geno)[2]))</pre>
> geno.obj <- SummarizedExperiment(geno)</pre>
> obj <- rqtClass(phenotype=pheno, genotype=geno.obj)</pre>
> res <- rQTest(obj, method="pca", out.type = "D")</pre>
> print(res)
Phenotype:
[1] 1 1 1 1 1 1
Genotype:
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1 0.9086994 0.9938212 0.9086994
$p.value
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                          p.Q3
      p.Q1
1 0.3404597 0.9285409 0.5654533
     Continuous phenotype
> library(rqt)
> data <- data.matrix(read.table(system.file("extdata/test.cont1.dat",</pre>
                                            package="rqt"), header = TRUE))
> pheno <- data[,1]</pre>
> geno <- data[, 2:dim(data)[2]]</pre>
> colnames(geno) <- paste(seq(1, dim(geno)[2]))</pre>
> geno.obj <- SummarizedExperiment(geno)</pre>
> obj <- rqtClass(phenotype=pheno, genotype=geno.obj)</pre>
> res <- rQTest(obj, method="pca", out.type = "C")</pre>
> print(res)
Phenotype:
[1] 3.422452 2.457394 2.708564 4.589394 5.461723 4.438707
Genotype:
    1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
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Covariates:
data frame with 0 columns and 0 rows
Results:
$Qstatistic
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                            QЗ
1 0.2846585 2.389537 2.219594
$p.value
                p.Q2
      p.Q1
                          p.Q3
1 0.593664 0.7022459 0.2561996
     Preprocessing with Partial Least Square regression (PLS)
This method is used for continous outcome, i.e. out.type = "C".
> library(rqt)
> data <- data.matrix(read.table(system.file("extdata/test.cont1.dat",</pre>
                                              package="rqt"), header = TRUE))
> pheno <- data[,1]</pre>
> geno <- data[, 2:dim(data)[2]]</pre>
> colnames(geno) <- paste(seq(1, dim(geno)[2]))</pre>
> geno.obj <- SummarizedExperiment(geno)</pre>
> obj <- rqtClass(phenotype=pheno, genotype=geno.obj)
> res <- rQTest(obj, method="pls", out.type = "C")</pre>
> print(res)
Phenotype:
[1] 3.422452 2.457394 2.708564 4.589394 5.461723 4.438707
Genotype:
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                            2
                               0
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                                      2
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                                                        1
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      1
[4,]
      0
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          1
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                               2
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[5,]
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      0
          2
                 1
                                                        1
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                                                                      1
                                                                          2
                                                                                  1
                                      2
                                          0
                                              1
                                                 0
                                                    0
[6,]
      2
              0
                 0
                     1
                        0
                            0
                               0
                                   0
                                                        0
                                                            0
                                                               0
. . .
Covariates:
data frame with 0 columns and 0 rows
```

Results:

```
$Qstatistic
                  Q2
         Q1
1 0.1910558 71.34471 16.59228
$p.value
                                p.Q3
       p.Q1
                    p.Q2
1 0.6620394 1.293491e-05 0.00011429
```

Preprocessing with Partial Least Square Discriminant Analysis (PLS-DA)

This method of data preprocessing is used for dichotomous outcome.

```
> library(rqt)
> data <- data.matrix(read.table(system.file("extdata/test.bin1.dat",</pre>
                                                  package="rqt"), header=TRUE))
> pheno <- data[,1]
> geno <- data[, 2:dim(data)[2]]</pre>
> colnames(geno) <- paste(seq(1, dim(geno)[2]))</pre>
> geno.obj <- SummarizedExperiment(geno)</pre>
```

```
> obj <- rqtClass(phenotype=pheno, genotype=geno.obj)
> res <- rQTest(obj, method="pls", out.type = "D", scale = TRUE)
      R2X(cum) R2Y(cum) Q2(cum) RMSEE pre ort pR2Y pQ2
Total
       0.0187
                  0.301 -0.292
                                  0.2
                                        1
                                            0 0.8 0.7
> print(res)
Phenotype:
[1] 1 1 1 1 1 1
Genotype:
     1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
[1,] 0 0 0 1 0 2 1 0 0
                              1
                                    0
                                         2
[2,] 1 0 1 0 0 1 0 0 0
                                             2
                                                          0
                        0
                           0
                              0
                                    0
                                       0
                                          0
                                                1
                                                       1
                                                             1
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                                 1
[3,] 0 0 0 0 1 0 0 1 0
                                    0
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                        1
                           1
                              1
                                 0
                                       0
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                                                          1
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                                                                   0
                                                       2
[4,] 0 0 1 0 1 0 0 1 1
                        0
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                              0
                                 1
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                                      0 0
                                            1
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                                                          0
                                                             1
[5,] 0 0 1 1 1 1 1 1 0
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[6,] 0 0 1 1 1 0 0 1 0
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                                                                0
     29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53
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[1,]
        0 0 0
                  0
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                        0
                           1
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[6,]
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     54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77
                  0
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[2,]
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[3,] 2
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[5,]
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[6,]
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     79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100
[1,]
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[2,]
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[3,]
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[6,]
               0
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                           0
                                          0
. . .
Covariates:
data frame with 0 columns and 0 rows
Results:
$Qstatistic
        Q1
                 Q2
```

1 11.12606 2.322913 11.12606

4.6 Using additional covariates

Quite often, researchers want to supply not only genetic data but also specific covariates, representic some physiological parameters or environment (for example, to evaluate hyphoteses of gene-environment interactions). In such cases, the package rqt can accept additional covariates, in form of N by K matrix, as provided below:

```
> library(rqt)
> data <- data.matrix(read.table(system.file("extdata/test.bin1.dat",</pre>
                                                package="rqt"), header = TRUE))
> pheno <- data[,1]
> geno <- data[, 2:dim(data)[2]]
> colnames(geno) <- paste(seq(1, dim(geno)[2]))</pre>
> geno.obj <- SummarizedExperiment(geno)</pre>
> covars <- read.table(system.file("extdata/test.cova1.dat",package="rqt"),</pre>
      header=TRUE)
> obj <- rqtClass(phenotype=pheno, genotype=geno.obj, covariates = covars)
> res <- rQTest(obj, method="pca", out.type = "D")</pre>
> print(res)
Phenotype:
[1] 1 1 1 1 1 1
. . .
Genotype:
     1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
[1,] 0 0 0 1 0 2 1 0 0
                         0
                            2
                               1
                                   2
                                      0
                                        0 2 1
                                                   1
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[2,] 1 0 1 0 0 1 0 0 0
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                         0
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                                0
                                   1
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[3,] 0 0 0 0 1 0 0 1 0
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[4,] 0 0 1 0 1 0 0 1 1
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[5,] 0 0 1 1 1 1 1 1 0
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[6,] 0 0 1 1 1 0 0 1 0
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     29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53
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[4,]
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     54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78
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[3,]
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[4,]
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[5,]
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[6,]
     0
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                      0 0 0
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                                      1 2
                                            1 1 0 0
                                                         0
                                                             0
                                                                0
         1
            1
                                                                   1
                                                                          1
     79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100
```

```
1 1 0 1 1 0 1
                       1 0 0 0 1 0
                       2 0
[2,]
                             2 1 0 0 2
                     1
                         0 2 0 0 0 0
                  0 2 0
[4,] 0 1
          2 0 1 0 1 2 0 0 0 0 0 1
                                                              0
[5,]
          0
            1
                0 0
                     2 0 0
                             0
                                0 1 0 0
[6,] 2 1 0 0 1 0 0 0 0 2 0 1 0 0
. . .
Covariates:
         COV1
1 -0.612463927
2 -0.464158885
3 0.006153597
4 -0.732109468
5 -0.223530136
6 -0.744903822
Results:
$Qstatistic
               Q2
1 2.012625 3.761859 2.012625
$p.value
      p.Q1
              p.Q2
                        p.Q3
1 0.1559952 0.8258796 0.2895166
    Meta-analysis
5
> library(rqt)
> data1 <- data.matrix(read.table(system.file("extdata/phengen2.dat",</pre>
```

```
package="rqt"), skip=1))
> pheno <- data1[,1]
> geno <- data1[, 2:dim(data1)[2]]</pre>
> colnames(geno) <- paste(seq(1, dim(geno)[2]))</pre>
> geno.obj <- SummarizedExperiment(geno)</pre>
> obj1 <- rqtClass(phenotype=pheno, genotype=geno.obj)
> data2 <- data.matrix(read.table(system.file("extdata/phengen3.dat",</pre>
                                                   package="rqt"), skip=1))
> pheno <- data2[,1]
> geno <- data2[, 2:dim(data2)[2]]</pre>
> colnames(geno) <- paste(seq(1, dim(geno)[2]))</pre>
> geno.obj <- SummarizedExperiment(geno)</pre>
> obj2 <- rqtClass(phenotype=pheno, genotype=geno.obj)</pre>
> data3 <- data.matrix(read.table(system.file("extdata/phengen.dat",</pre>
                                                   package="rqt"), skip=1))
> pheno <- data3[,1]
> geno <- data3[, 2:dim(data3)[2]]</pre>
> colnames(geno) <- paste(seq(1, dim(geno)[2]))</pre>
> geno.obj <- SummarizedExperiment(geno)</pre>
```

```
> obj3 <- rqtClass(phenotype=pheno, genotype=geno.obj)
> res.meta <- rQTestMeta(list(obj1, obj2, obj3))</pre>
> print(res.meta)
$final.pvalue
[1] 0.004276623
$pvalueList
[1] 0.000858092 0.367634896 0.245240026
$df
[1] 6
$chi.comb
[1] 18.93396
    Session information
> sessionInfo()
R version 3.2.4 (2016-03-10)
Platform: x86_64-apple-darwin13.4.0 (64-bit)
Running under: OS X 10.12.1 (unknown)
locale:
[1] C
attached base packages:
[1] stats4
              parallel stats
                                  graphics grDevices utils
                                                                datasets
[8] methods
              base
other attached packages:
 [1] rqt_0.99.0
                                SummarizedExperiment_1.0.2
 [3] Biobase_2.30.0
                                GenomicRanges_1.22.4
 [5] GenomeInfoDb_1.6.3
                                IRanges_2.4.8
 [7] S4Vectors_0.8.11
                                BiocGenerics_0.16.1
 [9] ropls_1.2.14
                                plyr_1.8.4
[11] pls_2.5-0
                                glmnet_2.0-5
[13] foreach_1.4.3
                                Matrix_1.2-7.1
loaded via a namespace (and not attached):
 [1] Rcpp_0.12.8
                                                              grid_3.2.4
                       lattice_0.20-34
                                           codetools_0.2-15
 [5] zlibbioc_1.16.0
                        CCP_1.1
                                           XVector_0.10.0
                                                              iterators_1.0.8
```

CompQuadForm_1.4.1

[9] tools_3.2.4

7 References

References

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- [6] Tibshirani, R. (1996) Regression shrinkage and selection via the lasso. J. Royal. Statist. Soc B., Vol. 58, No. 1, pages 267-288.