# Using RQT, an R 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 of rqt package

In order to install the rqt package, the user must first install R (http://www.r-project.org). After that, rqt can be installed with:

```
> source("http://bioconductor.org/biocLite.R")
> biocLite(c("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

```
> 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>
> 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:
     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:

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1 0.9086994 0.9938212 0.9086994
$p.value
                p.Q2
                          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]]
> 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="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
                 Q2
         Q1
1 0.2846585 2.389537 2.219594
$p.value
               p.Q2
                         p.Q3
     p.Q1
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)</pre>
> 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
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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
                    Q2
                              QЗ
          Q1
1 0.1910558 71.34471 16.59228
$p.value
```

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

This method of data preprocessing is used for dichotomous outcome.

p.Q2

1 0.6620394 1.293491e-05 0.00011429

p.Q1

```
R2X(cum) R2Y(cum) Q2(cum) RMSEE pre ort pR2Y pQ2
       0.0187
                0.301 -0.292
                               0.2
                                    1
                                         0 0.75 0.9
Total
<simpleError in model$scoreMN: $ operator not defined for this S4 class>
> 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
                                 0 0 2 1 1 0
[1,] 0 0 0 1 0 2 1 0 0 0
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[2,] 1 0 1 0 0 1 0 0 0
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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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    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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    79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100
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                              2 0 1 0 0
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[6,]
    2 1 0
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. . .
Covariates:
data frame with 0 columns and 0 rows
Results:
$Qstatistic
 Q1 Q2 Q3
1 NA NA NA
```

\$p.value

```
p.Q1 p.Q2 p.Q3
1 NA NA NA
```

### 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]</pre>
> geno <- data[, 2:dim(data)[2]]</pre>
> 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
                               2
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[2,] 1 0 1 0 0 1 0 0 0
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     79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95
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                                    0 1
                                          0 0 0
                                                    0
[6,] 2 1
           0 0 1 0 0 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.Q2
                           p.Q3
       p.Q1
1 0.1559952 0.8258796 0.2895166
    Meta-analysis
> 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)</pre>
> data2 <- data.matrix(read.table(system.file("extdata/phengen3.dat",</pre>
```

> pheno <- data2[,1]

> pheno <- data3[,1]

> geno <- data2[, 2:dim(data2)[2]]

> geno <- data3[, 2:dim(data3)[2]]

> colnames(geno) <- paste(seq(1, dim(geno)[2]))</pre>

> colnames(geno) <- paste(seq(1, dim(geno)[2]))</pre>

> obj2 <- rqtClass(phenotype=pheno, genotype=geno.obj)</pre>

> obj3 <- rqtClass(phenotype=pheno, genotype=geno.obj)
> res.meta <- rQTestMeta(list(obj1, obj2, obj3))</pre>

> data3 <- data.matrix(read.table(system.file("extdata/phengen.dat",

> geno.obj <- SummarizedExperiment(geno)</pre>

> geno.obj <- SummarizedExperiment(geno)</pre>

package="rqt"), skip=1))

package="rqt"), skip=1))

```
> print(res.meta)
$final.pvalue
[1] 0.01995627
$pvalueList
[1] 0.006016377 0.367634896 0.245240026
$df
[1] 6
$chi.comb
[1] 15.03891
    Session information
> sessionInfo()
R version 3.3.2 (2016-10-31)
Platform: x86_64-apple-darwin13.4.0 (64-bit)
Running under: macOS Sierra 10.12.1
locale:
[1] C/UTF-8/C/C/C
attached base packages:
[1] parallel stats4
                        stats
                                  graphics grDevices utils
```

[13] foreach\_1.4.3

base

[8] methods

[1] rqt\_0.99.3 SummarizedExperiment\_1.4.0
[3] Biobase\_2.34.0 GenomicRanges\_1.26.1
[5] GenomeInfoDb\_1.10.1 IRanges\_2.8.1
[7] S4Vectors\_0.12.1 BiocGenerics\_0.20.0
[9] ropls\_1.6.2 plyr\_1.8.4
[11] pls\_2.5-0 glmnet\_2.0-5

#### loaded via a namespace (and not attached):

[1] Rcpp\_0.12.8 lattice\_0.20-34 codetools\_0.2-15 grid\_3.3.2 [5] zlibbioc\_1.20.0 CCP\_1.1 XVector\_0.14.0 iterators\_1.0.8

Matrix\_1.2-7.1

datasets

[9] tools\_3.3.2 CompQuadForm\_1.4.2