# 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

```
> 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:
     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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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:
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Covariates:
data frame with 0 columns and 0 rows
Results:
$Qstatistic
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                  Q2
                            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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                 0
                     0
                        0
                            2
                               0
                                   0
                                      2
                                          0
                                              0
                                                        1
                                                            1
                                                                   0
      1
[4,]
      0
             2
                                      0
                                          0
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                                                 0
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                                                                                  0
          1
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                        0
                            1
                               2
                                   0
                                                        1
                                                            0
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                     1
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[5,]
              0
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                            2
                               0
                                   0
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                                          0
                                             1
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                                                               0
      0
          2
                 1
                                                        1
                                                                   1
                                                                      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)</pre>
> res <- rQTest(obj, method="pls", out.type = "D", scale = TRUE)
PLS-DA
200 \text{ samples } x \text{ } 100 \text{ variables and } 1 \text{ response}
standard scaling of predictors and response(s)
     R2X(cum) R2Y(cum) Q2(cum) RMSEE pre ort pR2Y pQ2
                 0.301 -0.292 0.2 1 0 0.95 0.8
<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
[1.] 0 0 0 1 0 2 1 0 0 0
                          2
                                   0
                                      0
                                        2
                                           2
[2,] 1 0 1 0 0 1 0 0 0
                       0
                          0
                             0
                                   0
                                      0
                                        0
                                              1
                                                 2
                                                       0
                                                                   0
                                1
                                                    1
                                                          1
                                                             0
                                                                0
[3,] 0 0 0 0 1 0 0 1 0
                       1
                          1
                             1
                                0
                                   0
                                      0 1 1
                                              0
                                                 0
                                                    1
                                                       1
                                                          1
                                                             0
                                                                0
                                                                   0
                                                                      1
[4,] 0 0 1 0 1 0 0 1 1
                                1
                                   0 0 0 1 0
                                                 0
                                                    2
                                                       0
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                                                                  0
                       0
                          0
                             0
                                                             0
[5,] 0 0 1 1 1 1 1 1 0
                       1
                          0
                             1
                                0
                                   0
                                     0 0 0 0
                                                 0
                                                    0
                                                       1
                                                          0
                                                             0
                                                                1
                                                                   0
[6,] 0 0 1 1 1 0 0 1 0
                      1
                          1
                             0
                                1
                                   0
                                     0 2 0 0
                                                 1
                                                    0
                                                       1
                                                          1
                                                             0
                                                                0
                                                                   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
[1,]
     1 0 0 0 0 0
                       0
                          1
                                0
                                   0
                                      1
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[2,]
     2 0 0
              0
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[4,]
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[5,]
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                                0
                                   2
                                    0 1 1
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[6,]
    0 0 0 0 1 0 1
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                                                                1
    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
     1 1 1 1 0 0 0
                         0
                                   0
                                                       2
[1,]
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[2,]
    2 1 0 1
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                                    2
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[3,] 2 1
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[4,]
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        1 1 0
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[5,]
    1
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                       2 1
                             1
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                                0
                                  1 2 1 1 0
                                                 0
[6,]
    0 1 1 0
                 0 0 0 0
                            0
                                                    0
                                                       0
                                                          0
                                                            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 1 0 1 1
                    0
                       1
                          1
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[2,]
        1 2 0
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     1
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[3,]
     1 1 0 0
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                                   0 0 0
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[4,]
        1
           2
              0
                 1
                    0
                       1
                          2
                             0
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[5,] 0 2
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                                2
                                   0
                                    1 0 0 0
[6,] 2 1
              0
                    0
                       0
                          0
                             0
                                                 0
. . .
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
                         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
                                               2
                                                    1
                                                           1
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                                                                 1
[3,] 0 0 0 0 1 0 0 1 0
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                                                           2
[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
[1,]
                   0
                      0
                         0
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                                1
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                                                                    1
[2,]
     2
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[3,]
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[4,]
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[5,]
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     0
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                                    0
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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 78
[1,]
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                                1
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[2,]
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[3,]
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```

```
0
                        2 1
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                                    0 0
                                          2
[5,]
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                           1
                              1
                                                                      1
                                 0
                                        2
     79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100
[1,]
                     0
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[2,]
           2 0
                  0
                              0
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                                    1
                                       0
                                          0
                                             2
                                                    0
                     0
                        1
                           2
[3.]
                  0
                     0
                        2
                           0
                              0
                                        0
           0
               0
[4,] 0 1
           2
               0
                  1
                     0
                        1
                           2
                              0
                                 0
                                    0 0
                                          0
                                             0
                                                    0
                                                       0
                                                          0
                                                                        0
[5,] 0 2 0 1
                  0 0
                        2 0
                              0
                                 0
                                    0 1 0 0
                                                 1
                                                    0
                                                       0
                                                          1
                                                             1
                                                                2 1
                                                                       1
[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
        Q1
                 Q2
1 2.012625 3.761859 2.012625
$p.value
                           p.Q3
       p.Q1
                 p.Q2
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]</pre>
> 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>
                                               package="rqt"), skip=1))
> pheno <- data2[,1]
> geno <- data2[, 2:dim(data2)[2]]
> colnames(geno) <- paste(seq(1, dim(geno)[2]))</pre>
```

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

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

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

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

```
> 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)</pre>
> res.meta <- rQTestMeta(list(obj1, obj2, obj3))</pre>
> 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
6
> 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
attached base packages:
[1] parallel stats4
                        stats
                                  graphics grDevices utils
                                                                  datasets
[8] methods
              base
other attached packages:
 [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
[13] foreach_1.4.3
                                Matrix_1.2-7.1
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
 [9] tools_3.3.2
```

CompQuadForm\_1.4.2

#### 7 References

#### References

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- [2] Li, B. and Leal, S.M. (2008) Methods for Detecting Associations with Rare Vari-ants for Common Diseases: Application to Analysis of Sequence Data. The American Journal of Human Genetics, 83, 311-321.
- [3] Liu D., Leal S. (2010) A Novel Adaptive Method for the Analysis of Next-Generation Sequencing Data to Detect Complex Trait Associations with Rare Variants Due to Gene Main Effects and Interactions, PLoS Genet., 6(10).
- [4] Madsen, B.E, Browning, S.R. (2009) A Groupwise Association Test for Rare Mutations Using a Weighted Sum Statistic, PLoS Genet., 5(2).
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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.