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

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December 7, 2016

## 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 two datasets: phenotype (a n by 1) matrix and genotype (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 <- read.table(system.file("extdata/test.bin1.dat",package="rqt"),</pre>
       header = TRUE)
> pheno <- data$pheno
> geno <- data[, 2:dim(data)[2]]</pre>
> obj <- rqtClass(phenotype=pheno, genotype=geno)
> res <- rQTest(obj, method="pca", out.type = "D")</pre>
> print(res)
Phenotype:
[1] 1 1 1 1 1 1
. . .
Genotype:
  snp1 snp2 snp3 snp4 snp5 snp6 snp7 snp8 snp9 snp10 snp11 snp12 snp13 snp14
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#### Covariates:

data frame with 0 columns and 0 rows

#### Results:

**\$Qstatistic** 

Q1 Q2 Q3 1 0.9086994 0.9938212 0.9086994

\$p.value

p.Q1 p.Q2 p.Q3 1 0.3404597 0.9285409 0.5654533

# 4.3 Continuous phenotype

```
> library(rqt)
> data <- read.table(system.file("extdata/test.cont1.dat",package="rqt"),
+ header = TRUE)
> pheno <- data$pheno
> geno <- data[, 2:dim(data)[2]]
> obj <- rqtClass(phenotype=pheno, genotype=geno)
> res <- rQTest(obj, method="pca", out.type = "C")
> print(res)
```

## Phenotype:

[1] 3.422452 2.457394 2.708564 4.589394 5.461723 4.438707

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Covariates:
data frame with 0 columns and 0 rows
Results:
$Qstatistic
         Q1
                   Q2
                             Q3
1 0.2846585 2.389537 2.219594
$p.value
                 p.Q2
      p.Q1
                            p.Q3
1 0.593664 0.7022459 0.2561996
      Preprocessing with Partial Least Square regression (PLS)
This method is used for continuous outcome, i.e. out.type = "C".
> library(rqt)
> data <- read.table(system.file("extdata/test.cont1.dat",package="rqt"),</pre>
      header = TRUE)
> pheno <- data$pheno
> geno <- data[, 2:dim(data)[2]]
> obj <- rqtClass(phenotype=pheno, genotype=geno)</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
Genotype:
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4         1         1         0         0         0         0         2         1         0         1         0         0         2           5         1         1         1         0         0         0         0         0         0         0         1         1         1           6         0         1         1         0         0         0         0         0         0         1			_	-				0						1
5         1         1         1         0         0         0         2         1         1         0         0         1         2         1           6         0         1         1         0         0         0         0         0         0         1         2         1           snp67         snp68         snp69         snp70         snp71         snp72         snp73         snp74         snp75         snp76         snp77         snp78         snp79           1         1         0         1         1         2         1         1         0         0         2         1         1         1         2         1         1         1         2         1         1         1         2         1         1         1         0         0         0         0         0         0         0         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         2         1         1         0         0         1         1         0         0			_	-				2						2
snp67         snp68         snp69         snp70         snp71         snp72         snp73         snp74         snp75         snp76         snp77         snp78         snp79           1         1         0         1         1         2         1         1         1         0         0         0         1         0         1         1         2         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1	_		_	-								0	-	1
1 1 0 1 0 1 1 2 1 1 1 0 0 0 2 1 1 1 1 2 1 1 1 0 0 0 2 1 1 1 1	6	0	1	1	0	0	0	0	0	0	0	1	2	1
2 1 0 1 0 1 0 1 0 0 0 0 0 0 1 1 2 1 2 1		snp67	snp68	snp69	snp70	snp71	snp72	snp73	snp74	snp75	snp76	snp77	snp78	snp79
3	1	1	0	1	1	2	1	1	1	0	0	2	1	1
4 1 0 0 1 1 1 1 0 0 0 0 0 0 0 0 1 0	2	1	0	1	-		0	0	0	0		1	2	1
5  0  0  1  1  0  0  1  1  0  0  1  0  1  0  1  0  1  0  1  0  1  0  6  1  0  6  1  0  0  0  0  0  0  0  1  1  1  1  1	3		0									_	-	1
6	4		0	-	_	_	1	0	0		-	0	_	0
snp80         snp81         snp82         snp83         snp84         snp85         snp86         snp87         snp88         snp89         snp90         snp91         snp92           1         1         0         1         1         0         0         0         1         0         0         1         0         0         1         0         0         1         0         1         0         0         1         0         0         1         0         0         0         2         0	_	-	0	_	_	-	0	1	1	_	-	1	_	0
1 1 0 1 1 0 1 1 0 0 1 1 0 0 0 0 1 0 1 2 0 2 1 0 0 0 2 3 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	O	_	•	-	•	-	•	_	_	_	_	_	_	2 2nn92
2 1 2 0 0 0 1 2 0 2 1 0 0 0 2 3 1 0 0 0 2 3 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	1	_	_	_	_	_	_	_	_	_	_	_	_	
4 1 2 0 1 0 1 2 0 0 0 0 0 0 0 0 5 2 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	_				_				0		-	_	0	2
5 2 0 1 0 0 2 0 0 0 0 1 0 0 0 0 0 0 0 0 0	3	1	0	0	0	0	2	0	0	2	0	0	0	0
6 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	4	1	2	0	1	0	1	2	0	0	0	0	0	0
snp93       snp94       snp95       snp96       snp97       snp98       snp99       snp100         1       1       0       0       1       0       1       1         2       1       0       0       0       1       1       2         3       1       1       0       0       1       2       0       0         4       1       0       0       1       0       0       0         5       1       0       0       1       1       1       1								0					0	
1     1     0     0     0     1     0     1     1       2     1     0     0     0     1     1     2       3     1     1     0     0     1     2     0     0       4     1     0     0     0     1     0     0     0       5     1     0     0     1     1     1     1     1	6										0	1	0	0
2     1     0     0     0     0     1     1     2       3     1     1     0     0     1     2     0     0       4     1     0     0     0     1     0     0     0       5     1     0     0     1     1     2     1     1			_						_					
3     1     1     0     0     1     2     0     0       4     1     0     0     0     1     0     0     0       5     1     0     0     1     1     2     1     1														
4     1     0     0     0     1     0     0       5     1     0     0     1     1     2     1     1														
5 1 0 0 1 1 2 1 1														
•••														

Covariates:

data frame with 0 columns and 0 rows

```
Results:
$Qstatistic
         Q1
                  Q2
1 0.1910558 71.34471 16.59228
$p.value
                     p.Q2
                                p.Q3
       p.Q1
1 0.6620394 1.293491e-05 0.00011429
      Preprocessing with Partial Least Square Discriminant Analysis (PLS-DA)
This method of data preprocessing is used for dichotomous outcome.
> library(rqt)
> data <- read.table(system.file("extdata/test.bin1.dat",package="rqt"),</pre>
      header=TRUE)
> pheno <- data$pheno
> geno <- data[, 2:dim(data)[2]]</pre>
> obj <- rqtClass(phenotype=pheno, genotype=geno)
> res <- rQTest(obj, method="pls", out.type = "D", scale = TRUE)
      R2X(cum) R2Y(cum) Q2(cum) RMSEE pre ort pR2Y pQ2
Total
                  0.301 -0.292
        0.0187
                                   0.2
                                          1
> print(res)
Phenotype:
[1] 1 1 1 1 1 1
. . .
Genotype:
1
          0
               0
                               2
                                    1
2
     1
          0
               1
                     0
                          0
                               1
                                    0
                                          0
                                                     0
                                                            0
                                                                  0
                                                                         1
3
               0
                     0
     0
          0
                          1
                               0
                                    0
                                          1
                                               0
                                                      1
                                                            1
                                                                  1
                                                                         0
```

snp1 snp2 snp3 snp4 snp5 snp6 snp7 snp8 snp9 snp10 snp11 snp12 snp13 snp14 snp15 snp16 snp17 snp18 snp19 snp20 snp21 snp22 snp23 snp24 snp25 snp26 snp27 snp28 snp29 snp30 snp31 snp32 snp33 snp34 snp35 snp36 snp37 snp38 snp39 snp40 

6	0	0	0	0	0	1	0	1	1	0	0	2	0
	snp41	snp42	snp43	snp44	snp45	snp46	snp47	snp48	snp49	snp50	snp51	snp52	snp53
1	0	0	1	0	0	0	0	1	1	0	0	0	1
2	1	0	0	1	0	0	0	2	0	1	0	1	0
3	0	1	0	1	0	2	1	1	1	0	0	0	1
4	0	0	1	1	0	0	1	1	2	0	1	0	2
5	2	2	0	0	0	1	2	1	0	0	0	0	1
6	1	1	2	0	0	0	1	1	1	0	0	1	1
	snp54	snp55	snp56	snp57	snp58	snp59	snp60	snp61	snp62	snp63	snp64	snp65	snp66
1	1	1	1	1	0	0	0	0	1	1	0	1	1
2	2	1	0	1	0	0	0	1	0	1	2	2	1
3	2	1	0	0	0	0	0	0	0	1	0	0	1
4	1	1	0	0	0	0	2	1	0	1	0	0	2
5	1	1	1	0	0	0	2	1	1	0	0	1	1
6	0	1	1	0	0	0	0	0	0	0	1	2	1
			snp69									snp78	
1	1	0	1	1	2	1	1	1	0	0	2	1	1
2	1	0	1	0	1	0	0	0	0	1	1	2	1
3	1	0	1	2	1	0	1	1	0	0	0	0	1
4	1	0	0	1	1	1	0	0	0	0	0	1	0
5	0	0	1	1	0	0	1	0	1	0	0	1	0
6	1	0	0	0	0	0	1	1	1	1	1	1	2
			snp82										
1	1	0	1	1	0	1	1	0	0	0	1	0	1
2	1	2	0	0	0	1	2	0	2	1	0	0	2
3	1	0	0	0	0	2	0	0	2	0	0	0	0
4	1	2	0	1	0	1	2	0	0	0	0	0	0
5	2	•	1	0	0	2	0	0	2	0	1	0	0
6	_	0	•	_	0	0	0	0		0	1	0	0
1	snp93	snp94	snp95	snp96	snp97	sup98	snp99	_	) 1				
_	1	0	0	0	0	1	1	-	2				
2	1	1	0	0		2	0		2				
4	1	0	0	0	1 1	0	0	(	) )				
5	1	0	0	1	1	2	1		1				
6	0	0	0	1	1	0	1		1				
U	U	U	U	1	1	U	1	•	L				

Covariates:

data frame with 0 columns and 0 rows

Results:

\$Qstatistic

Q1 Q2 Q3 1 11.12606 2.322913 11.12606

\$p.value

p.Q1 p.Q2 p.Q3 1 0.0008512339 0.0008512339 0.0008512339

## 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(rgt)
> data <- read.table(system.file("extdata/test.bin1.dat",package="rqt"),</pre>
      header = TRUE)
> pheno <- data$pheno
> geno <- data[, 2:dim(data)[2]]
> covars <- read.table(system.file("extdata/test.cova1.dat",package="rqt"),</pre>
       header=TRUE)
> obj <- rqtClass(phenotype=pheno, genotype=geno, covariates = covars)
> res <- rQTest(obj, method="pca", out.type = "D")</pre>
> print(res)
Phenotype:
[1] 1 1 1 1 1 1
Genotype:
  snp1 snp2 snp3 snp4 snp5 snp6 snp7 snp8 snp9 snp10 snp11 snp12 snp13 snp14
                 0
                       1
                             0
                                   2
                                              0
                                                    0
                                                           0
           0
                                        1
                                                                  2
1
2
                 1
                       0
                             0
                                   1
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     1
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3
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                       0
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4
                 1
                       0
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                                              1
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5
     0
           0
                 1
                       1
                             1
                                   1
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                                               1
                                                    0
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                                                                  0
                                                                         1
                                                                                0
6
                 1
                       1
                             1
                                   0
                                        0
                                               1
                                                    0
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                                                                  1
                                                                                1
                                                                                       0
        snp16 snp17 snp18 snp19 snp20 snp21 snp22 snp23 snp24 snp25 snp26 snp27
  snp15
              2
                            1
                                   0
                                         0
                                                 1
                                                        0
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1
                    1
                    2
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2
      0
              0
                                   2
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3
      0
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4
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                                                                                    2
6
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       0
                            0
                                   1
                                                 1
                                                        1
                                                                                           0
  snp28
        snp29 snp30 snp31 snp32 snp33 snp34 snp35 snp36 snp37 snp38 snp39 snp40
                    0
                            0
                                   0
                                         0
                                                 0
                                                        0
                                                              1
2
       1
              2
                    0
                            0
                                   0
                                         1
                                                 2
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                                                                                           0
3
       0
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                    0
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                                                 1
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4
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       2
              0
                    0
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                                   1
                                                 2
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                                                                                           0
                                                                      1
                                                                             1
5
       0
              0
                    0
                            0
                                   0
                                          1
                                                        0
                                                              0
6
                    0
                                   0
                                                 0
                                                                                    2
       0
              0
                            0
                                         1
                                                        1
                                                               1
                                                                      0
                                                                             0
                                                                                           0
  snp41
         snp42 snp43 snp44 snp45 snp46 snp47 snp48 snp49
                                                                 snp50 snp51
                                                                               snp52 snp53
      0
              0
                            0
                                   0
                                         0
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2
              0
                    0
                                   0
                                         0
                                                 0
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                                                                                           0
       1
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                                         2
3
      0
                    0
                                   0
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              1
                            1
                                                 1
                                                        1
                                         0
                                                               2
4
       0
              0
                    1
                            1
                                   0
                                                 1
                                                        1
                                                                      0
                                                                             1
                                                                                    0
                                                                                           2
       2
              2
                                                 2
5
                    0
                            0
                                   0
                                         1
                                                        1
                                                              0
                                                                      0
                                                                                           1
6
                    2
                            0
                                   0
                                         0
                                                              1
                                                                      0
                                                                             0
                                                                                    1
                                                                                           1
       1
              1
                                                 1
                                                        1
  snp54 snp55 snp56 snp57 snp58 snp59 snp60 snp61 snp62 snp63 snp64 snp65 snp66
                    1
                            1
                                   0
                                         0
                                                 0
                                                        0
                                                              1
```

2	2	1	0	1	0	0	0	1	0	1	2	2	1
3	2	1	0	0	0	0	0	0	0	1	0	0	1
4	1	1	0	0	0	0	2	1	0	1	0	0	2
5	1	1	1	0	0	0	2	1	1	0	0	1	1
6	0	1	1	0	0	0	0	0	0	0	1	2	1
	snp67	snp68	snp69	snp70	snp71	snp72	snp73	snp74	snp75	snp76	snp77	snp78	snp79
1	1	0	1	1	2	1	1	1	0	0	2	1	1
2	1	0	1	0	1	0	0	0	0	1	1	2	1
3	1	0	1	2	1	0	1	1	0	0	0	0	1
4	1	0	0	1	1	1	0	0	0	0	0	1	0
5	0	0	1	1	0	0	1	0	1	0	0	1	0
6	1	0	0	0	0	0	1	1	1	1	1	1	2
	snp80	snp81	snp82	snp83	snp84	snp85	snp86	snp87	snp88	snp89	snp90	snp91	snp92
1	1	0	1	1	0	1	1	0	0	0	1	0	1
2	1	2	0	0	0	1	2	0	2	1	0	0	2
3	1	0	0	0	0	2	0	0	2	0	0	0	0
4	1	2	0	1	0	1	2	0	0	0	0	0	0
5	2	0	1	0	0	2	0	0	0	0	1	0	0
6	1	0	0	1	0	0	0	0	2	0	1	0	0
	snp93	snp94	snp95	snp96	snp97	snp98	snp99	snp100	)				
1	1	0	0	0	1	0	1		l				
2	1	0	0	0	0	1	1	2	2				
3	1	1	0	0	1	2	0	(	)				
4	1	0	0	0	1	0	0	(	)				
5	1	0	0	1	1	2	1	:	L				

## Covariates:

COV1

- 1 -0.612463927
- 2 -0.464158885
- 3 0.006153597
- 4 -0.732109468
- 5 -0.223530136
- 6 -0.744903822

# Results:

## \$Qstatistic

Q1 Q2 Q3 1 2.012625 3.761859 2.012625

# \$p.value

p.Q1 p.Q2 p.Q3 1 0.1559952 0.8258796 0.2895166

# 5 Meta-analysis

```
> library(rqt)
> data1 <- read.table(system.file("extdata/phengen2.dat",package="rqt"), skip=1)</pre>
> obj1 <- rqtClass(phenotype=data1[,1], genotype=data1[, 2:dim(data1)[2]])</pre>
> data2 <- read.table(system.file("extdata/phengen3.dat",package="rqt"), skip=1)</pre>
> obj2 <- rqtClass(phenotype=data2[,1], genotype=data2[, 2:dim(data2)[2]])</pre>
> data3 <- read.table(system.file("extdata/phengen.dat",package="rqt"), skip=1)</pre>
> obj3 <- rqtClass(phenotype=data3[,1], genotype=data3[, 2:dim(data3)[2]])</pre>
> 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] stats
             graphics grDevices utils datasets methods
                                                                 base
other attached packages:
[1] rqt_0.99.0
                  ropls_1.2.14
                                  plyr_1.8.4
                                                 pls_2.5-0
                                                                 glmnet_2.0-5
[6] foreach_1.4.3 Matrix_1.2-7.1
loaded via a namespace (and not attached):
[1] CCP_1.1
                     parallel_3.2.4 tools_3.2.4
                                                              Rcpp_0.12.8
[5] codetools_0.2-15 grid_3.2.4
                                                              CompQuadForm_1.4.1
                                          iterators_1.0.8
[9] lattice_0.20-34
```

# 7 References

# References

- [1] Wu, M. C., Lee, S., Cai, T., Li, Y., Boehnke, M., and Lin, X. (2011) Rare Variant Association Testing for Sequencing Data Using the Sequence Kernel Association Test (SKAT). The American Journal of Human Genetics, 89, 82-93.
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- [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.