Introduction to GWAS using R and GenABEL

LUPA Workshop in Statistical Methods for GWAS studies

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What is R?

R is a:

- programming language
- software environment

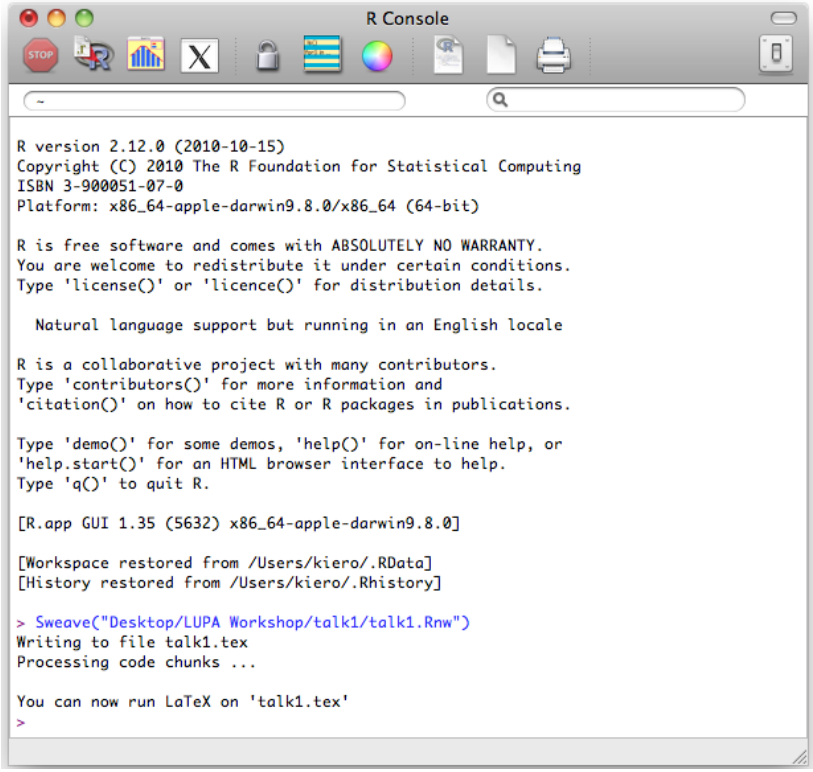
for:

- statistical computing
- beautiful graphics

- *de facto* standard among statisticians
- widely used for development and data analysis
- an implementation of the S programming language
- partially inspired by Scheme
- created by Ross Ihaka and Robert Gentleman at the University of Auckland, New Zealand
- source code is freely available under the GNU GPL
- mainly command line
- GUIs exist
- many tools/tests at hand
- project homepage: http://www.r-project.org

R code example

```
> 2 + 2
[1] 4
> p.value <- 0.05
> p.value
[1] 0.05
> -log10(p.value)
[1] 1.30103
> print("Hello!")
[1] "Hello!"
```



Power of R

R is modular – there is a core and you can load packages containing custom functions.

- 2984 packages available on CRAN (02.05.2011) – http://cran.r-project.org/
- 998 projects registered on R-Forge (02.05.2011) – http://r-forge.r-project.org/
- 460 packages available on BioConductor (03.05.2011) – http://www.bioconductor.org/
- from genetics to social sciences and from geology to cryptography

Installing packages

```
> install.packages("GenABEL")
> install.packages("DatABEL",
+ repos="http://R-Forge.R-project.org")
```

Loading packages

```
> require("GenABEL") # Within functions
> library("GenABEL")
```

How to get help

```
> vignette("GenABEL") # Package level
> demo(graphics)
> help(qtscore)         # Function level
> ?qtscore
> ??qtscore                  # Extensive search
```

Function for generating random genotypes

```
> generateGenotypes <- function(num.markers = 1, missing = F) {
+         if (missing) {
+                 genotypes <- sample(1:5, num.markers, replace = T)
+         }
+         else {
+                 sample(1:4, num.markers, replace = T) -> genotypes
+         }
+         genotypes[genotypes == 1] <- "A"
+         genotypes[genotypes == 2] <- "T"
+         genotypes[genotypes == 3] <- "C"
+         genotypes[genotypes == 4] <- "G"
+         genotypes[genotypes == 5] <- "X"
+         genotypes
+ }
```

```
> generateGenotypes(5)
[1] "T" "T" "G" "G" "G"
> generateGenotypes(num.markers = 5)
[1] "G" "T" "A" "G" "G"
> generateGenotypes(10, T)
 [1] "C" "C" "A" "T" "A" "A" "G" "A" "G" "A"
> generateGenotypes(missing = T, num.markers = 10)
 [1] "G" "A" "X" "A" "A" "C" "X" "A" "A" "C"
```

GenABEL project – http://www.genabel.org

The mission of the GenABEL project is to provide a framework for collaborative, sustainable, transparent, open-source based development of statistical genomics methodology. We aim to streamline methodology discussion, development, implementation, dissemination and maintenance; through the community.

GenABEL is developed by a Team led Dr. Yurii Aulchenko, Erasmus MC, Rotterdam.

- **GenABEL** – genome-wide association analysis for quantitative, binary and time-till-event traits.
- **MetABEL** – meta-analysis of genome-wide SNP association results GWAS for quantitative, binary and time-till-event trait.
- **ProbABEL** – genome-wide association analysis of imputed data.
- **PredictABEL** – assess the performance of risk models for binary outcomes.
- **DatABEL** – file-based access to large matrices stored on HDD in binary format.
- **ParallABEL** – generalized parallelization of GWAS.
- **MixABEL** – more mixed models GWAS; experimenting with GSL, multiple input formats, iterator, parallelization through threads.

Data representation in GenABEL

- ***.raw** – genotype data GenABEL internal binary format.
- ***.dat** – phenotype data, e.g., as in PLINK.

Binary format = compression, e.g. for 170K SNP chip 200 individuals: data.ped – 144.4MB vs. data.raw – 32.9MB.

*.dat file format

```
    id sex age bt1 ct ct1
"289982" 0 30.33 NA NA 3.93
"325286" 0 36.514 1 0.49 3.61
"357273" 1 37.811 0 1.65 5.30
```

Importing from different data formats

- `convert.snp.text` – convert from text format.
- `convert.snp.ped` – convert from PED format.
- `convert.snp.tped` – convert from TPED format.
- `convert.snp.illumina` – convert from Illumina format.

Loading data

```
> data <- load.gwaa.data("dataset/phenotype.dat",
+          "dataset/genotype.raw",
+          makemap = T)
```

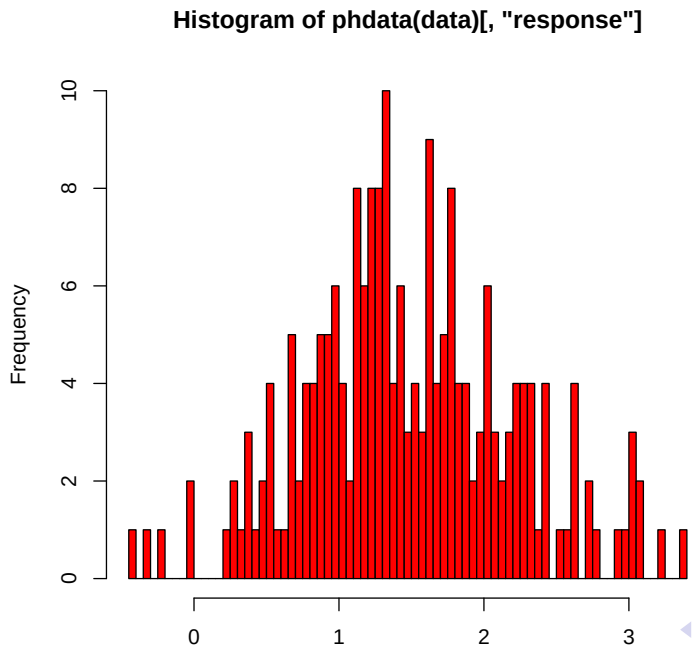
If coords are chromosome specific, you can make them genome-wise by: `makemap = T`.

Examine the phenotype data

```
> nids(data)
[1] 207
> nsnps(data)
[1] 174375
> phdata(data)[2,]
           id sex bt       ct group response
dog225 dog225   1  0 1.925575     3 1.569402
> phdata(data)[1:5, "sex"]
[1] 1 1 0 1 0
```

Get summary for trait response

```
> summary(phdata(data)[,"response"])
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.    NA's
-0.4325  0.9996  1.4090  1.4860  1.9480  3.3860  1.0000
> hist(phdata(data)[,"response"],
+ breaks = 100,
+ col = "red")
```



Examine the genotype data 1st individual, markers 3-5

```
> gtdata(data)[1,3:5]
@nids = 1
@nsnps = 3
@nbytes = 1
@idnames = dog224
@snpnames = BICF2P1383091 TIGRP2P259 BICF2P186608
@chromosome = 1 1 1
@coding =  04 01 01
@strand =  00 00 00
@map = 3212349 3249189 3265742
@male = 1
@gtps =
80 40 40
```

Get summary for markers 2 and 3

```
> summary(gtdata(data))[2:3,]
                 Chromosome Position Strand A1 A2 NoMeasured CallRate Q.2 P.11
BICF2G630707846           1  3082514      u  1  2        206 0.995169 0.0  206
BICF2P1383091             1  3212349      u  A  G        207 1.000000 0.5    0
                 P.12 P.22       Pexact Fmax         Plrt
BICF2G630707846    0    0 1.000000e+00    0 1.000000e+00
BICF2P1383091    207    0 1.240547e-61   -1 2.282010e-64
```

Is the binary trait bt correlated with sex?

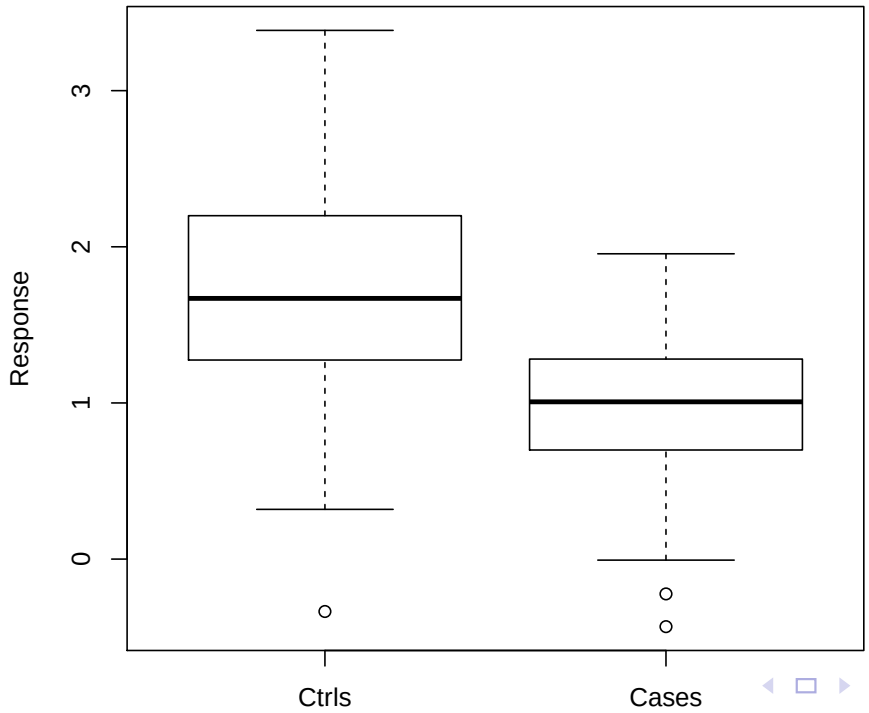
```
> tab <- table(phdata(data)$bt, phdata(data)$sex)
> fisher.test(tab)

        Fisher's Exact Test for Count Data

data:  tab
p-value = 1
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 0.5322721 1.9110534
sample estimates:
odds ratio
  1.010358
```

Is the binary trait bt related to response?

```
> boxplot(phdata(data)$response ~ phdata(data)$bt,
+ names = c("Ctrls", "Cases"),
+ ylab = "Response")
```



Do a simple QC?

```
> qc1 <- check.marker(data, call = 0.95,
+ perid.call = 0.95,
+ maf = 1e-08,
+ p.lev = 1e-08)
> ...
> data.clean <- data[qc1$idok, qc1$snpok]
```

Do a simple association test

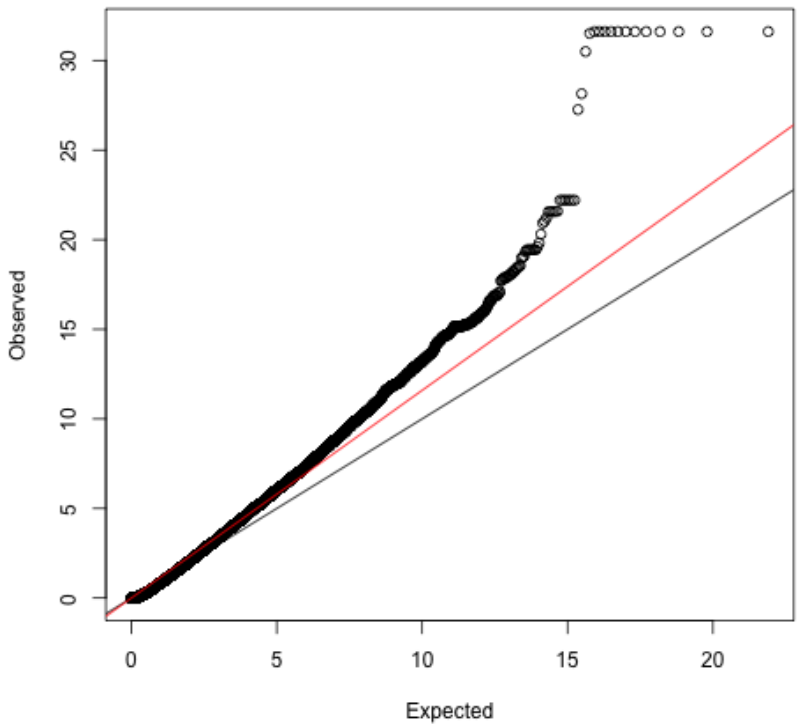
```
> an <- qtscore(bt ~ response, data,
+ trait.type="binomial", times=1)
> summary(an, top=5)

Summary for top 5 results, sorted by P1df
                 Chromosome  Position Strand A1 A2   N         effB      se_effB
BICF2P506952              1  90475257      u  A  G 204 -0.002009766 0.0003574521
BICF2G630348662           3 339590471      u  T  C 204 -0.002009766 0.0003574521
TIGRP2P51678              3 339878416      u  C  T 204 -0.002009766 0.0003574521
BICF2G630348969           3 340378977      u  T  G 204 -0.002009766 0.0003574521
BICF2P628966              3 340641323      u  C  T 204 -0.002009766 0.0003574521
                 chi2.1df          P1df effAB effBB chi2.2df          P2df
BICF2P506952    31.61223 1.882407e-08     0    NA 31.61223 1.882407e-08
BICF2G630348662 31.61223 1.882407e-08     0    NA 31.61223 1.882407e-08
TIGRP2P51678    31.61223 1.882407e-08     0    NA 31.61223 1.882407e-08
BICF2G630348969 31.61223 1.882407e-08     0    NA 31.61223 1.882407e-08
BICF2P628966    31.61223 1.882407e-08     0    NA 31.61223 1.882407e-08
                       Pc1df
BICF2P506952    1.767672e-07
BICF2G630348662 1.767672e-07
TIGRP2P51678    1.767672e-07
BICF2G630348969 1.767672e-07
BICF2P628966    1.767672e-07
```

What is λ, show Q-Q plot...

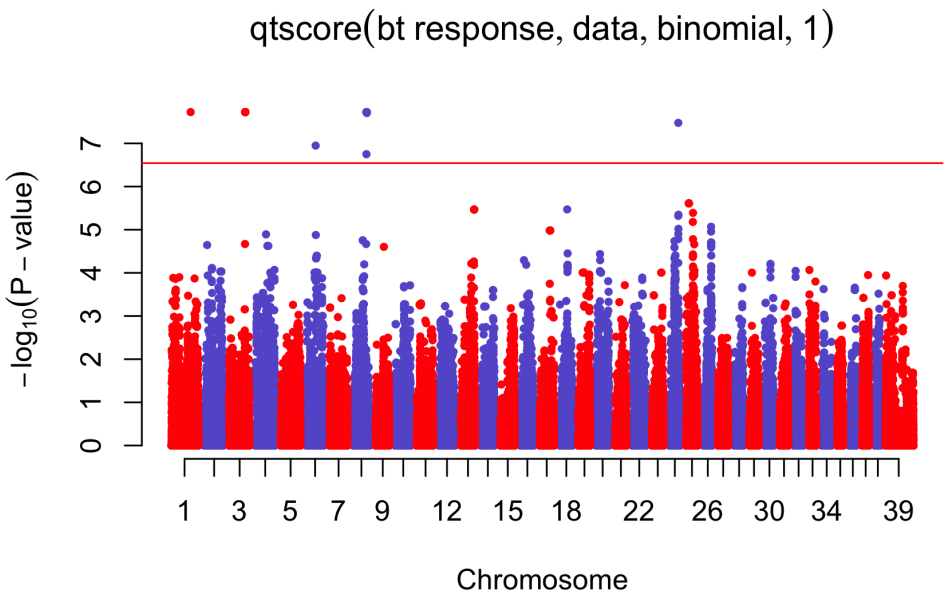
```
> estlambda(an[,"P1df"])
$estimate
[1] 1.159153

$se
[1] 0.0003918843
```



What about a Manhattan plot?

```
> plot(an,
+ col = c("red", "slateblue"),
+ pch = 19,
+ cex = .5,
+ df = "1")
> bonferroni <- -log10(0.05 / nsnps(data))
> abline(h=bonferroni, col = "red")
```



GenABEL – why?
Easiness of comparing different approaches...

Load your data one time and enjoy:

- Simple association tests.
- Genomic control.
- PCA-based correction – Eigenstrat.
- Mixed models.
- Structured association.
- Any combination of the above!

The END

Thank You! and:

- Leif Andersson
- Yurii Aulchenko
- Örjan Carlborg
- Dirk Jan de Koonig
- Kerstin Lindblad-Toh
- Xia Shen
- Katarina Tengvall

Practical information

Use account:

- login: Kurs_LUPAonStatistic
- password: LupaStat2011
- DO NOT TRY TO LOGIN TO VMWARE (Windows) - you will block the whole account!!!

Website:

http://www.computationalgenetics.se/LUPA2011