

# GenABEL tutorial

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# Chapter 1

## Overview

**GenABEL-package** is an R library developed to facilitate Genome-Wide Association (GWA) analysis of binary and quantitative traits. **GenABEL-package** is implemented as an R library. R is a free, open source language and environment for general-purpose statistical analysis (available at <http://www.r-project.org/>). It implements powerful data management and analysis tools. Though it is not strictly necessary to learn everything about R to run **GenABEL-package**, it is highly recommended as this knowledge will improve flexibility and quality of your analysis.

Originally **GenABEL-package** was developed to facilitate GWA analysis of quantitative traits using data coming from extended families and/or collected from genetically isolated populations. At the same time **GenABEL-package** implements a large number of procedures used in analysis of population-based data; it supports analysis of binary and quantitative traits, and of survival (time-to-event) data. Most up-to-date information about **GenABEL-package** can be found at the web-site <http://mga.bionet.nsc.ru/nlru/GenABEL/>.

**GenABEL-package** is a part of more extensive ABEL collection (<http://mga.bionet.nsc.ru/~yurii/ABEL/>) of software supporting different kinds of GWA analyses.

This tutorial was originally written to serve as a set of exercises for the "Advances in population-based studies of complex genetic disorders" (GE03) course of the Netherlands Institute of Health Sciences (Nihes).

If you read this tutorial not as a part of the GE03 course, and you are eager to start with your GWA analysis without reading all the not-so-strictly-necessary stuff, start directly from the section 5 ("Genome-wide association analysis").

Otherwise, you can start with R basics and simple association analyses using few SNPs in section 2, "Introduction to R". In the next section, 4 ("Introduction to **GenABEL-package**") you will learn how to work with the `gwaa.data-class`, which is used to store GWA data in **GenABEL-package** and will perform some simple large-scale analyses.

In the next section, 5 ("Genome-wide association analysis"), you will do quality control of genetic data and do association analysis under realistic conditions. This section is the core of this tutorial.

The section 7 ("GWA in presence of genetic stratification: practice") is dedicated to analysis in presence of populational stratification and analysis of family-based data.

Genetic data imputations are covered in section ??, "??".

The last section, 11 ("Analysis of selected region"), is dedicated to analysis of haplotype association and analysis of SNP interactions.

Information on importing the data from different formats to **GenABEL-package** is given in appendix A ("Importing data to **GenABEL-package**"). Answers to exercises are provided at the end of respective chapters.

Experienced R users start directly with the section (4, "Introduction to **GenABEL-package**").



## Chapter 2

# Introduction to R

In this section we will consider base R data types and operations, and tools for analysis of qualitative and quantitative traits. Only basic R functionality – the things which are crucial to know before we can proceed to genetic association analysis – will be covered within this section. If you want to make most of your data, though, we strongly recommend that you improve your knowledge of R using books other than this. A number of excellent manuals ('An introduction to R', 'Simple R', 'Practical Regression and Anova using R', and others) is available free of charge from the R project web-site (<http://www.r-project.org>).

In the first part of this chapter you will learn about the most important R data types and will learn how to work with R data. Next, we will cover exploratory data analysis. The chapter will end with introduction to regression analysis.

### 2.1 Basic R data types and operations

On the contrast to many other statistical analysis package, analysis in R is not based on graphic user interface, but is command line-based. When you first start R, a command prompt appears. To get help and overview of R, type `help.start()` on the command line and press **enter**. This will start internet browser and open the main page of the R documentation.

Let us first use R as a powerful calculator. You can directly operate with numbers in R. Try multiplying two by three:

```
> 2*3
```

```
[1] 6
```

Other standard arithmetic operations can be performed in similar manner:

```
> 2/3
```

```
[1] 0.6666667
```

(division)

```
> 2^3
```

```
[1] 8
```

```
(power)
```

```
> 2-3
```

```
[1] -1
```

```
(subtraction)
```

```
> 2+3
```

```
[1] 5
```

```
(summation)1.
```

Mathematical functions, such as square roots, base-10 logarithm, and exponentiation, are available in R as well:

```
> sqrt(5)
```

```
[1] 2.236068
```

```
> log10(2.24)
```

```
[1] 0.350248
```

```
> exp(0.35)
```

```
[1] 1.419068
```

Here, we have computed  $e$  to the power of base-10 logarithm of the square root of the sum of two and three. After each operation performed, we have rounded the result to the two digits after the floating point – just in order to do less typing.

The arithmetic operations and functions can be nested and therefore we can obtain the above result in one line, and without the 2nd-digit approximation:

```
> exp(log10(sqrt(2+3)))
```

```
[1] 1.418337
```

R functions include not only the standard mathematical ones, but also a wide range of statistical function, for example, probability density functions of many probability distributions. We will make extensive use of these at a later stage, when computing significance and estimating statistical power.

For any function with name say '`fun`', help may be obtained by typing '`help(fun)`' (or '`?fun`') on the command line.

R help pages have standard layout, documenting usage of the function, explaining function arguments, providing details of implementation and/or usage, explaining the value returned by the function, and giving references and examples of the function use.

---

<sup>1</sup>For complete list of arithmetic operations try `help("+")`

Most of the documented functions have examples of their usage at the end of the 'help' page, and these examples can be evaluated in R. E.g. try `'example(log10)'`.

### Exercise 1. Explore help for Wilcoxon test

Explore the help page for the Wilcoxon test (function: `wilcox.test`) and answer the questions:

1. When exact Wilcoxon test is computed by default?
2. If the default conditions for the exact test are not satisfied, what approximation is used?

If you do not know the exact name for the function you look for, try `'help.search("query")'`, where `query` is the keyword.

### Exercise 2. Finding functions and help pages

Try to find out what are the functions to do

1. Fisher exact test
2. T-test

One of important R operations is *assignment*, which is done with '`<-`' operator. A (new) variable name should be provided on the left-hand side of this operator and on the right-hand side, there must be either name of already existing variable or an expression. For example, we if want to assign value '2' to variable 'a', and value '3' to the variable 'b' we would use the assignment operator:

```
> a <- 2
> b <- 3
```

Typing the variable name in R command line will return its' value, e.g.

```
> b
[1] 3
```

Evaluation of the expression

```
> exp(log10(sqrt(a+b)))
[1] 1.418337
```

gives the expected result we have obtained above using numerical arguments.

While the variables 'a' and 'b' contain single numeric values, variables in general can be multi-dimensional; an one-dimensional example of such is a vector (array). Let us create an example vector and experiment with it:

```
> v <- c(1,3,5,7,11)
```

Here, '`c()`' is a function, which combines its arguments to make a vector. This vector is then assigned to a variable named 'v'.

Now, let us try different operations with this vector:

```
> v + 1
```

```
[1] 2 4 6 8 12
```

It is easy to see that the result is a vector, which is obtained by adding one to each element of the original vector `v`. Other arithmetic operations and mathematical functions behave in the same way, e.g. the operation is performed for each element of the vector, and the results are returned:

```
> 1/v
```

```
[1] 1.00000000 0.33333333 0.20000000 0.14285714 0.09090909
```

```
> log(v)
```

```
[1] 0.000000 1.098612 1.609438 1.945910 2.397895
```

What happens if two vectors are supplied as function arguments? Let us define a new vector

```
> ov <- c(1,2,3,4,5)
```

and add it to the vector `v`:

```
> v + ov
```

```
[1] 2 5 8 11 16
```

You can see that the summation was done element-wise, i.e. the first element of the result vector is obtained as the sum of the first elements of `v` and `ov`, the second is the sum of the second elements, and so forth.

Other arithmetic operations with two vectors are performed in the same element-wise manner:

```
> v * ov
```

```
[1] 1 6 15 28 55
```

(multiplication)

```
> v^ov
```

```
[1] 1 9 125 2401 161051
```

(power).

The vector operations considered above returned a same-length vector as output. There are others – statistical and summary – functions which evaluate a vector as a whole and return a single value as output. For example, to obtain a sum of vector's elements, use

```
> sum(v)
```

```
[1] 27
```

Other examples of such functions involve `length`, returning number of elements of a vector, `mean`, returning the mean, `var`, returning the variance, etc.:

```
> length(v)
```

```
[1] 5
```

```
> mean(v)
```

```
[1] 5.4
```

```
> var(v)
```

```
[1] 14.8
```

One of the basic, and probably most used, data operations in R is *sub-setting*.

This refers to an operations which help you deriving a subset of the data. Let us create a short vector and play a bit with sub-setting. This vector will contain 5 simple character strings:

```
> a <- c("I am element 1","I am element 2","I am element 3","I am element 4","I am element 5")
> a
```

```
[1] "I am element 1" "I am element 2" "I am element 3" "I am element 4"
[5] "I am element 5"
```

To find out what is the value of the  $i$ -th element of this vector, you can sub-set it by `a[i]`. For example the 3rd elements is:

```
> a[3]
```

```
[1] "I am element 3"
```

You can also select a bigger sub-set, e.g. all elements from 2 to 4:

```
> a[c(2:4)]
```

```
[1] "I am element 2" "I am element 3" "I am element 4"
```

Here, operation `c(2:4)` stays for 'combine numbers from 2 to 4 into a vector'. An equivalent result is obtained by

```
> a[c(2,3,4)]
```

```
[1] "I am element 2" "I am element 3" "I am element 4"
```

We can also easily get disjoint elements; e.g. if you want to retrieve elements 1, 3, and 5, you can do that with

```
> dje <- c(1,3,5)
```

```
> dje
```

```
[1] 1 3 5
```

```
> a[dje]
```

```
[1] "I am element 1" "I am element 3" "I am element 5"
```

One of very attractive features of R data objects is possibility to derive a subset based on some condition. Let us consider two vectors, `tmphgt`, containing the height of some subjects, and `tmpids`, containing their identification codes (IDs):

```
> tmphgt <- c(150,175,182,173,192,168)
> tmphgt

[1] 150 175 182 173 192 168

> tmpids <- c("fem1","fem2","man1","fem3","man2","man3")
> tmpids

[1] "fem1" "fem2" "man1" "fem3" "man2" "man3"
```

Imagine you need to derive the IDs of the people with height over 170 cm. To do that, we need to combine several operations. First, we should run the logical function `>170` on the height data:

```
> vec <- (tmphgt>170)
> vec

[1] FALSE TRUE TRUE TRUE TRUE FALSE
```

This returns a logical vector whose elements are 'TRUE', when a particular element of the `tmphgt` satisfies the condition `>170`. The returned logical vector, in turn, can be applied to sub-set any other vector of the same length<sup>2</sup>, including itself. Thus if you need to see what are the heights in people, which are taller than 170 cm, you can use

```
> tmphgt[vec]

[1] 175 182 173 192
```

As you can see, only the elements of `tmphgt`, for which the corresponding value of `vec` was 'TRUE', are returned. In the same manner, the logical vector `vec` can be applied to select elements of the vector of IDs:

```
> tmpids[vec]

[1] "fem2" "man1" "fem3" "man2"
```

You can combine more than one logical condition to derive sub-sets. For example, to see what are the IDs of people taller than 170 but shorter than 190 cm, you can use

```
> vec <- (tmphgt>170 & tmphgt<190)
> vec

[1] FALSE TRUE TRUE TRUE FALSE FALSE
```

---

<sup>2</sup> Actually, you can apply it to a longer vector too, and then the logical vector will be "expanded" to total length by repeating the original vector head-to-tail. However, we will not use this in our exercises.

```
> tmpids[vec]
```

```
[1] "fem2" "man1" "fem3"
```

A better<sup>3</sup> way to do logical sub-setting assumes use of the `which()` function on the top of the logical vector. This function reports which elements are `TRUE`. To obtain above results you can run:

```
> vec <- which(tmpght>170 & tmpght<190)
> vec
```

```
[1] 2 3 4
```

```
> tmpids[vec]
```

```
[1] "fem2" "man1" "fem3"
```

You can see that `vec` contains a vector, whose elements are the indexes of the elements of `tmpght` for which the logical condition satisfies.

Sub-setting for 2D objects (matrices) is done in similar manner. Let us construct a simple matrix and do several sub-setting operations on it:

```
> a <- matrix(c(      11,12,13,
+                21,22,23,
+                31,32,33
+                ),nrow=3,ncol=3)
> a
```

```
      [,1] [,2] [,3]
[1,]   11   21   31
[2,]   12   22   32
[3,]   13   23   33
```

To obtain the element in the 2nd row and 2nd column, you can use

```
> a[2,2]
```

```
[1] 22
```

To access the element from the second row and third column, use

```
> a[2,3]
```

```
[1] 32
```

Note that here, the row index (2) comes first, and the column index (3) comes second.

To obtain the 2x2 set of elements contained in upper left corner, you can do

```
> a[1:2,1:2]
```

```
      [,1] [,2]
[1,]   11   21
[2,]   12   22
```

---

<sup>3</sup> Because it treats NAs for you

	1	2	3
1	1	4	7
2	2	5	8
3	3	6	9

Table 2.1: Vector representation of a matrix. Elements in the table are the vector indices of the matrix elements.

Or you can even get the variables, which reside in corners:

```
> a[c(1,3),c(1,3)]
```

```
      [,1] [,2]
[1,]   11   31
[2,]   13   33
```

If one of the dimensions is not specified, complete vector is returned for this dimension. For example, here we retrieve the first row

```
> a[1,]
```

```
[1] 11 21 31
```

...and the third column

```
> a[,3]
```

```
[1] 31 32 33
```

...or columns 1 and 3:

```
> a[,c(1,3)]
```

```
      [,1] [,2]
[1,]   11   31
[2,]   12   32
[3,]   13   33
```

Other way to address elements of a matrix is to use one-dimensional index. For example, if you want to access element in the 2nd row and 2nd column, instead of

```
> a[2,2]
```

```
[1] 22
```

you can use

```
> a[5]
```

```
[1] 22
```



This way of accessing the elements of a matrix is based on the fact, that each matrix can be preseted as a vector, whose elements are numbered consecutively: the element in the upper-left corner has index 1, the element in the second row of the first column has index 2, and the last elemnt in the borrom-right corner has the maximal value, as shown in Table 2.1.

As well as with vectors, you can sub-set matrices using logical conditions or indexes. For example, if we want to see what elements of `a` are greater than 21, we can run

```
> a>21

      [,1] [,2] [,3]
[1,] FALSE FALSE TRUE
[2,] FALSE  TRUE TRUE
[3,] FALSE  TRUE TRUE
```

or, better

```
> which(a>21)

[1] 5 6 7 8 9
```

Note that in the latter case, a vector whose elements give the 1-D indexes of the matrix, is returned. This vector indicates the elemnts of matrix `a`, for which the condition (`a>21`) is satisfied.

You can obtain the values of the matrix's elements, for which the condition isfulfilled, either by

```
> a[a>21]

[1] 22 23 31 32 33
```

or

```
> a[which(a>21)]

[1] 22 23 31 32 33
```

Once again, the latter method should be preferred. Consider an example, where some elements of the matrix are missing (`NA`) – a situation which is common in real data analysis. Let us replace the elemnt number 5 with `NA` and perform sub-setting operations on the resulting matrix:

```
> a

      [,1] [,2] [,3]
[1,]  11   21   31
[2,]  12   22   32
[3,]  13   23   33

> a[5] <- NA
> a
```

```
      [,1] [,2] [,3]
[1,]   11   21   31
[2,]   12   NA   32
[3,]   13   23   33
```

```
> a[a>21]
```

```
[1] NA 23 31 32 33
```

```
> a[which(a>21)]
```

```
[1] 23 31 32 33
```

You can see that when `a[a>21]` was used, not only the elements which are greater than 21 were returned, but also `NA` was. As a rule, this is not what you want, and `which` should be used unless you do want to make some use of the `NA` elements.

In this section, we have generated a number of R data objects. Some of these were numeric (e.g. vector of heights, `tmphgt`) and some were character, or string (e.g. vector of study IDs, `tmpids`). Some times you need to figure out what is the class of a certain object. This can be done using the `class()` function. For example,

```
> tmphgt
```

```
[1] 150 175 182 173 192 168
```

```
> class(tmphgt)
```

```
[1] "numeric"
```

```
> tmpids
```

```
[1] "fem1" "fem2" "man1" "fem3" "man2" "man3"
```

```
> class(tmpids)
```

```
[1] "character"
```

What happens if we try to find out the class of

```
> a
```

```
      [,1] [,2] [,3]
[1,]   11   21   31
[2,]   12   NA   32
[3,]   13   23   33
```

– an object, which contains a matrix?

```
> class(a)
```

```
[1] "matrix"
```

Results are expected – we find out that `a` is a matrix, which is correct. At the same time, a matrix is an upper-level class, which contains a number of elements, belonging to some lower-level (e.g. character/numeric) class. To see what is the class of the matrix's elements, try

```
> a[1,]

[1] 11 21 31

> class(a[1,])

[1] "numeric"
```

which says that elements (at least of the first row) are numeric. Because all elements of a matrix should have the same class, we can conclude that `a` is a matrix containing numeric values.

At this point, it is worthwhile inspecting what data objects were created during our work. This can be done with the `ls()` command:

```
> ls()

[1] "a"      "b"      "dje"    "old"    "ov"     "tmphgt" "tmpids" "v"
[9] "vec"
```

Obviously, this "list" command is very useful – you will soon find that it is just too easy to forget the name of a variable which it took long time to create. Some times you may wish to remove some of the data objects because you do not need them anymore. You can remove an object using the `rm()` command, where the names of objects to be deleted are listed as arguments. For example, to remove `tmphgt` and `tmpids` variable you can use

```
> rm(tmphgt,tmpids)
```

If you now look up what data objects are still left in your workspace with the `ls()` command

```
> ls()

[1] "a"      "b"      "dje"    "old"    "ov"     "v"      "vec"
```

you find that you have successfully deleted `tmphgt` and `tmpids`.

At this point, you can exit R by typing `q()` on the command line and pressing **Enter**.

### Summary:

- You can get access to the top-level R documentation by `help.start()` command. To search help for some keyword `keywr`, you can use `help.search(keywr)` command. To get description of some function `fun`, use `help(fun)`.
- You can use R as a powerful calculator

- It is possible to get sub-sets of vectors and matrices by specifying index value or a logical condition (of the same length as the vector / matrix) between square brackets (`[, ]`)
- When you obtain an element of a matrix with `[i,j]`, `i` is the row and `j` is the column of the matrix.
- Function `which(A)` returns index of the elements of `A` which are `TRUE`
- You can see objects available in your workspace by using the `ls()` command
- Unnecessary object (say, `tmphgt`) can be deleted from the workspace using `rm` command, e.g. `rm(tmphgt)`
- You can leave R using the `q()` command

### Exercise 3. Exploring `srdta`

In this exercise, you will explore few vectors representing different data on study subjects described in `srdta` example data set supplied together with `GenABEL-package`. First, you need to load `GenABEL-package` by typing

```
> library(GenABEL)
```

and load the data by

```
> data(srdta)
```

The vector containing study subjects sex can be accessed through `male(srdta)`; this vector's value is one when the corresponding person is male and zero otherwise. The vector containing SNP names can be accessed via `snpname(srdta)`, chromosome ID – through `chromosome(srdta)` and map – through `map(srdta)`. Explore these vectors and answer the questions.

1. What is the ID and sex of the first person in the data set?
2. Of the 22nd person?
3. How many males are observed among first hundred subjects?
4. How many FEMALES are among 4th hundred?
5. What is the male proportion in first 1000 people?
6. What is the FEMALE proportion in second 1000 (1001:2000) people?
7. What is name, chromosome and map position of 33rd maker?
8. What is distance between markers 25 and 26?

## 2.2 Data frames

A *data frame* is a class of R data, which, basically, is a data table. In such tables, it is usually assumed that rows correspond to subjects (observations) and

columns correspond to variables (characteristics) measured on these subjects. A nice feature of data frames is that columns (variables) have names, and the data can be addressed by referencing to these names<sup>4</sup>.

We will explore R data frames using example data set `assoc`. Start R with double-click on the file named `assocbase.RData`. You can see the names of the loaded objects by using the "list" command:

```
> ls()

[1] "assoc"
```

Thus, only one object is loaded. The class of this object is:

```
> class(assoc)

[1] "data.frame"
```

– a data frame.

The dimensionality of a data frame (or a matrix) can be determined by using the `dim()` command:

```
> dim(assoc)

[1] 250  7
```

Here, the first number corresponds to the number of rows (subjects) and the second to the number of columns (variables). Thus, the data frame `assoc` contains the data on 250 subjects, who are characterised by 7 variables each.

Let us now figure out what are the names of the 7 variables present in the data frame. To see what are the variable names, use the command `names()`:

```
> names(assoc)

[1] "subj" "sex"  "aff"  "qt"   "snp4" "snp5" "snp6"
```

These variables correspond to the personal identifier (ID, variable `subj`), sex, affection status, quantitative trait `qt` and several SNPs. Each variable can have its own type (numeric, character, logic), but all variables must have the same length – thus forming a matrix-like data structure.

A variable from a data frame (say, `fram`), which has some name (say, `nam`) can be accessed through `fram$nam`. This will return a conventional vector, containing the values of the variable. For example to see the affection status (`aff`) in the data frame `assoc`, use

```
> assoc$aff

[1] 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0
[26] 0 1 0 0 1 1 0 0 0 0 0 0 0 0 1 0 0 1 NA 0 0 0 0 0
[51] 1 1 0 1 0 0 0 0 0 0 1 0 1 0 0 0 0 0 0 1 0 0 0 0
[76] 0 0 1 1 1 0 0 0 0 0 0 0 0 1 1 0 0 0 0 1 NA 0 0 0 0
```

---

<sup>4</sup>This may also be true for matrices; more fundamental difference is though that a matrix *always* contains variables of the same data type, e.g. character or numeric, while a data frame may contain variables of different types

```

[101] 1 0 0 1 1 0 1 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 1 0
[126] 1 0 0 0 1 0 0 0 1 0 1 NA 1 0 0 0 0 0 0 0 0 0 1 0 0
[151] 0 0 0 0 0 0 0 1 1 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0
[176] 0 0 1 1 NA 0 0 0 0 1 1 1 1 0 NA 1 0 0 0 0 0 0 1 1 0
[201] 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 1 0 0 0 0 1 0 0 0
[226] 0 0 0 1 1 0 0 0 0 0 1 0 0 0 0 1 0 0 0 1 0 0 0 0

```

The `aff` (affected) variable here codes for a case/control status, conventionally, the cases are coded as "1" and controls as "0". You can also see several "NA"s, which stays for missing observation.

#### Exercise 4. Exploring `assoc`

1. Investigate types of the variables presented in data frame `assoc`. For each variable, write down the class.

Data frame may be thought of as a matrix which is a collection of (potentially different-type) vectors. All sub-setting operations discussed before for matrices are applicable to a data frame, while all operations discussed for vectors are applicable to data frame's variables.

Thus, as any particular variable present in a data frame is a conventional vector, its elements can be accessed using the vector's indices. For example, if you would like to know what are the ID, sex and affection status for the person with index 75, you can request

```
> assoc$subj[75]
```

```
[1] 1409
```

```
> assoc$sex[75]
```

```
[1] 1
```

```
> assoc$aff[75]
```

```
[1] 0
```

Alternatively, using the matrix-style of sub-setting, you can see all the data for person 75:

```
> assoc[75,]
```

```

      subj sex aff      qt snp4 snp5 snp6
75 1409   1   0 1.014664 A/B  B/A  B/B

```

In the same manner as with matrices, you can get data for e.g. subjects 5 to 15 by

```
> assoc[5:15,]
```

	subj	sex	aff	qt	snp4	snp5	snp6
5	1533	0	0	0.1009220	A/B	B/A	B/A
6	2466	1	0	-0.1724321	A/B	A/A	A/A
7	2425	0	0	-0.3378473	B/B	A/A	A/A
8	1068	0	0	-1.7112925	A/A	B/B	<NA>
9	198	1	0	-0.4815822	A/B	B/A	B/A
10	1496	1	0	1.2281232	A/A	B/B	B/B
11	909	0	0	0.5993945	A/B	B/A	B/A
12	1213	0	0	1.9792190	A/A	B/B	B/B
13	181	1	0	1.5435921	A/A	B/B	B/B
14	1783	0	0	-1.6242738	A/B	B/A	B/A
15	1914	0	0	-0.5160331	A/A	B/B	B/B

The result is actually a new data frame containing data only on people with index from 5 to 15:

```
> x<-assoc[5:15,]
> class(x)

[1] "data.frame"

> dim(x)

[1] 11  7
```

As well as with matrices and vectors, it is possible to sub-set elements of a data frame based on (a combination of) logical conditions. For example, if you are interested in people who have the `qt` values over 1.4, you can find out what are the indices of these people

```
> vec <- which(assoc$qt>1.4)
> vec

[1] 12 13 33 41 54 68 72 76 89 106 118 142 156 161 175 181 193 219 241
```

and then show the complete data with

```
> assoc$subj[vec]

[1] 1213 181 1737 1319 516 1355 186 1426 1284 822 2129 212 1443 704 1648
[16] 1628 562 858 698
```

At the same time, if you only want to check what are the IDs of these people, try

```
> assoc$subj[vec]

[1] 1213 181 1737 1319 516 1355 186 1426 1284 822 2129 212 1443 704 1648
[16] 1628 562 858 698
```

Or, if we are interested to find what are the IDs and what are the SNP genotypes of these people, we can try

```
> assoc[vec,c(1,5,6,7)]
```

	subj	snp4	snp5	snp6
12	1213	A/A	B/B	B/B
13	181	A/A	B/B	B/B
33	1737	A/A	B/B	B/B
41	1319	A/A	B/A	B/A
54	516	A/B	B/A	B/A
68	1355	A/A	B/B	B/B
72	186	A/A	B/A	B/A
76	1426	A/B	B/A	B/A
89	1284	A/A	B/B	B/B
106	822	A/B	B/A	B/A
118	2129	A/B	B/A	B/A
142	212	A/B	B/A	B/A
156	1443	A/A	B/B	B/B
161	704	A/B	B/A	B/A
175	1648	A/B	B/A	B/A
181	1628	A/B	B/A	B/A
193	562	A/A	B/B	B/B
219	858	A/B	B/A	B/A
241	698	B/B	A/A	A/A

here, we select people identified by `vec` in the first dimension (subjects), and by `c(1,5,6,7)` we select first, fifth, sixth and seventh column (variable).

The same result can be obtained using variables' names insted of the variables' indices. To remind you the variables' names:

```
> names(assoc)

[1] "subj" "sex"  "aff"  "qt"   "snp4" "snp5" "snp6"
```

And now make a vector of the variables' names of interest and filter the data based on it:

```
> namstoshow <- c("subj", "snp4", "snp5", "snp6")
> assoc[vec, namstoshow]
```

	subj	snp4	snp5	snp6
12	1213	A/A	B/B	B/B
13	181	A/A	B/B	B/B
33	1737	A/A	B/B	B/B
41	1319	A/A	B/A	B/A
54	516	A/B	B/A	B/A
68	1355	A/A	B/B	B/B
72	186	A/A	B/A	B/A
76	1426	A/B	B/A	B/A
89	1284	A/A	B/B	B/B
106	822	A/B	B/A	B/A
118	2129	A/B	B/A	B/A
142	212	A/B	B/A	B/A
156	1443	A/A	B/B	B/B
161	704	A/B	B/A	B/A



```
175 1648 A/B B/A B/A
181 1628 A/B B/A B/A
193 562 A/A B/B B/B
219 858 A/B B/A B/A
241 698 B/B A/A A/A
```

A more convenient way to access data presented in a data frame is through "attaching" it to the R search path by

```
> attach(assoc)
```

After that, the variables can be accessed directly, e.g.

```
> subj[75]
```

```
[1] 1409
```

instead of `assoc$subj[75]`.

While it is possible to explore the data presented in a data frame using the sub-setting operations and screen output, and modify certain data elements using the assignment ("`<-`") operation, you can also explore and modify the data contained in a data frame<sup>5</sup> by using `fix()` command (e.g. try `fix(assoc)`). However, normally this is not necessary.

With attached data frames, a possible complication is that later on you may have several data frames which contain the variables with the same names. The variable which will be used when you directly use the name would be the one from the data frame attached last. You can use `detach()` function to remove a certain data frame from the search path, e.g. after

```
> detach(assoc)
```

we can not use direct reference to the name (try `subj[75]`) anymore, but have to use the full path instead:

```
> assoc$subj[75]
```

```
[1] 1409
```

### Summary:

- The list of available objects can be viewed with `ls()`; a class of some object `obj` can be interrogated with `class(obj)`.
- Simple summary statistics for numeric variables can be generated by using `summary` function
- Histogram for some variable `var` can be generated by `hist(var)`
- A variable with name `name` from a data frame `frame`, can be accessed through `frame$name`.

---

<sup>5</sup>and also a matrix

- You can attach the data frame to the search path by `attach(frame)`. Then the variables contained in this data frame may be accessed directly. To detach the data frame (because, e.g., you are now interested in other data frame), use `detach(frame)`.

### Exercise 5. Explore phenotypic part of `srdta`

Load the `srdta` data object supplied with GenABEL by loading the package with `library(GenABEL)` and then loading the data with `data(srdta)`. The `srdta` object contains a data frame with phenotypes. This data frame may be accessed through `phdata(srdta)`. Explore this data frame and answer the questions

1. What is the value of the 4th variable for the subject number 75?
2. What is the value of variable 1 for person 75? Check what is the value of this variable for the first ten people. Can you guess what first variable is?
3. What is the sum of variable 2? Can you guess what data variable 2 contains?

## 2.3 Exploratory analysis of qualitative and quantitative traits

Let us now attach the data frame `assoc`

```
> attach(assoc)
```

and explore it.

Let us first check how many of the subjects are males. In the `sex` variable, males are coded with "1" and females with "0". Therefore to see the number of males, you can use

```
> sum(sex==1)
```

```
[1] 129
```

and to determine what is male sex proportion you can use

```
> sum(sex==1)/length(sex)
```

```
[1] 0.516
```

This way to compute the proportion would only work correctly if there are no missing observations (`length()` returns the total length of a variable, including NAs).

Because of the way the males are coded, the same answer is reached by

```
> mean(sex)
```

```
[1] 0.516
```

However, that would not have worked if the sex was coded differently, e.g. with "1" for males and "2" for females.

Let us now try to find out the mean of the quantitative trait `qt`. By definition, the mean of a variable, say  $x$  (with  $i$ -th element denoted as  $x_i$ ) is

$$\bar{x} = \frac{\sum_{i=1}^N x_i}{N}$$

where  $N$  is the number of measurements.

If we try to find out the mean of `qt` by direct use of this formula, we first need to find out the sum of the `qt`'s elements. The `sum()` function of R precisely does the operation we need. However, if we try it

```
> sum(qt)
```

```
[1] NA
```

this returns "NA". The problem is that the `qt` variable contains "NA"s (try `qt` to see these) and by default the "NA" is returned. We can, however, instruct the `sum()` function to remove "NA"s from consideration:

```
> sum(qt, na.rm=T)
```

```
[1] -26.4733
```

where `na.rm=T` tells R that missing variables should be removed (NonAvailable.ReMove=True)<sup>6</sup>.

We can now try to compute the mean with

```
> sum(qt, na.rm=T)/length(qt)
```

```
[1] -0.1058932
```

This result, however, is not correct. The `length()` function returns the total length of a vector, which includes "NA"s as well. Thus we need to compute the number of the `qt`'s elements, which are not missing.

For this, we can use R function `is.na()`. This function returns `TRUE` if supplied argument is missing (NA) and `FALSE` otherwise. Let us apply this function to the vector `assoc$qt`:

```
> is.na(qt)
```

```
[1] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[13] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE TRUE FALSE FALSE FALSE
[25] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[37] TRUE FALSE FALSE FALSE FALSE FALSE TRUE FALSE FALSE FALSE FALSE FALSE
[49] FALSE TRUE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[61] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[73] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
```

---

<sup>6</sup>The same argument works for a number of R statistical functions such as `mean`, `median`, `var`, etc

```

[85] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[97] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[109] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[121] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE TRUE FALSE FALSE FALSE FALSE
[133] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[145] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[157] FALSE FALSE FALSE FALSE FALSE FALSE TRUE FALSE FALSE FALSE FALSE FALSE FALSE
[169] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[181] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[193] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE TRUE FALSE FALSE
[205] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[217] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[229] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[241] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE

```

Indeed, the 7 missing elements are correctly identified. However, we are interested in elements which are **not** missing. To get these, we can use the logical function NOT (!), which changes all FALSE to TRUE and visa versa:

```

> !is.na(qt)

 [1] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
[13] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE FALSE TRUE TRUE TRUE
[25] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
[37] FALSE TRUE TRUE TRUE TRUE TRUE TRUE FALSE TRUE TRUE TRUE TRUE TRUE
[49] TRUE FALSE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
[61] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
[73] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
[85] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
[97] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
[109] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
[121] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE FALSE TRUE TRUE TRUE TRUE
[133] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
[145] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
[157] TRUE TRUE TRUE TRUE TRUE TRUE FALSE TRUE TRUE TRUE TRUE TRUE TRUE
[169] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
[181] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
[193] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE FALSE TRUE
[205] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
[217] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
[229] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
[241] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE

```

Thus the number of elements which are not missing<sup>7</sup> is

```

> sum(!is.na(qt))

[1] 243

```

Finally, we can compute the mean of the qt with

---

<sup>7</sup>A hidden trick here is that arithmetic operations treat TRUE as one and FALSE as zero

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```
> sum(qt, na.rm=T)/sum(!is.na(qt))
```

```
[1] -0.1089436
```

While this way of computing the mean is enlightening in the sense of how to treat the missing values, the same correct result should be normally achieved by supplying the `na.rm=T` argument to the `mean()` function:

```
> mean(qt, na.rm=T)
```

```
[1] -0.1089436
```

The function `table(x)` produces a frequency table for the variable `x`. Thus, we can use

```
> table(sex)
```

```
sex
 0  1
121 129
```

which, again, tells us that there are 129 males and 121 females in this data set. This function excludes missing observations from consideration.

Tables of other qualitative variables, such as affection and SNPs, can be generated in the same manner.

As with arithmetic operations and mathematical functions, most of the R operations can be combined within a single line. Let us try to combine logical conditions and the `table()` command to check the distribution of number of affected in men and women separately:

```
> table(aff[which(sex==1)])
```

```
 0  1
95 31
```

```
> table(aff[which(sex==0)])
```

```
 0  1
95 24
```

On R command line pressing the "up-arrow" button makes the last typed command re-appear (pressing it one more time will bring you to the one before the last, so on). This is very handy when you have to repeat the same analysis of different variables

#### Exercise 6. Explore assoc

Explore phenotypic variables presented in `assoc`

1. How many affected and unaffected are present in the data set?

2. What is the proportion of affected?
3. What is the distribution of `snp4` (how many different genotype classes are present and what are the counts)?

Contingency tables for pairs of variables (cross-tables) can be generated in R using the `table` command we have used in previous section to explore frequency distributions. For example, if you want cross-tabulate sex and affection status in the data frame `assoc`, you can use

```
> table(sex,aff)
```

```
      aff
sex  0  1
  0 95 24
  1 95 31
```

Here, the first variable (`sex`) is presented in rows and the second (affection status) in columns.

As is usually the case with R, the output may be saved as a new object (of class 'table', which is a variety of a matrix):

```
> a<-table(sex,aff)
> class(a)
```

```
[1] "table"
```

```
> a
```

```
      aff
sex  0  1
  0 95 24
  1 95 31
```

and this object may be analysed further.

For example, we can easily get the number of affected male with

```
> a[2,2]
```

```
[1] 31
```

Alternatively, we can analyse the resulting contingency table `a` with more complex functions. If we want to see proportions in this table, we can use

```
> prop.table(a)
```

```
      aff
sex      0      1
  0 0.38775510 0.09795918
  1 0.38775510 0.12653061
```

Needless to say, this is equivalent to

```
> prop.table(table(assoc$sex,assoc$aff))
```

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```

      0      1
0 0.38775510 0.09795918
1 0.38775510 0.12653061

```

In the above table, we see what proportion of people belong to four different classes (affected male, affected female, unaffected male and unaffected female). We may be though interested in the proportion of males in affected and unaffected. This may be achieved by

```
> prop.table(a,2)
```

```

      aff
sex      0      1
0 0.5000000 0.4363636
1 0.5000000 0.5636364

```

saying us that 56.4% of affected are male.

Alternatively, we may be interested in proportion of affected among males/females. To answer this question, run

```
> prop.table(a,1)
```

```

      aff
sex      0      1
0 0.7983193 0.2016807
1 0.7539683 0.2460317

```

saying us that 56.4% of male are affected.

Other useful contingency table analysis function is `fisher.test`, which implements the Fisher Exact Test of independence:

```
> fisher.test(a)
```

```
Fisher's Exact Test for Count Data
```

```

data:  a
p-value = 0.4457
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 0.676759 2.482869
sample estimates:
odds ratio
 1.290313

```

Exploration of genetic data within base R, though possible, may be a bit of a pain. For example, we can easily generate contingency table of SNP5 vs affected status:

```
> a <- table(aff,snp5)
> a
```

```

      snp5
aff A/A B/A B/B
0   31  88  68
1    8  26  17

```

We can also look up what is the proportion of affected among different genotypic groups

```
> prop.table(a,2)

      snp5
aff      A/A      B/A      B/B
0 0.7948718 0.7719298 0.8000000
1 0.2051282 0.2280702 0.2000000
```

showing that proportion of cases is similar in 'A/A' and 'A/B' genotypic groups and somewhat decreased in 'B/B'. It is easy to test if this affection is statistically independent of genotype by

```
> chisq.test(a)

Pearson's Chi-squared test
```

```
data:  a
X-squared = 0.2511, df = 2, p-value = 0.882
```

which gives (insignificant) genotypic association test on two degrees of freedom.

However, testing Hardy-Weinberg equilibrium, testing allelic effects, and even computation of allelic frequency is not so straightforward. Such specific genetic tests are implemented in special R libraries, such as **genetics** and **GenABEL-package** and will be covered in later sections of this document.

At this moment we will switch to exploratory analysis of quantitative traits. We will make use of the **srdta** data supplied with **GenABEL-package**. As you can remember from an exercise, the library is loaded with `library(GenABEL)` and the data are loaded with `data(srdta)`: Then the phenotypic data frame may be accessed through `phdata(srdta)`.

### Exercise 7. Explore phenotypes in **srdta**

Explore phenotypic data content of **srdta** object (`phdata(srdta)`).

1. How many observations and variables are presented in the data frame?
2. What are the classes of these variables?

As it was mentioned before, the function `summary()` generates a summary statistics for an object. For example, to see summary for trait **qt1**, we can use

```
> summary(phdata(srdta)$qt1)

      Min. 1st Qu.  Median    Mean 3rd Qu.    Max.    NA's
-4.6000 -0.9500 -0.3100 -0.2981  0.3800  3.2000      3
```

`summary` is quite useful function which may operate in different ways for objects of different classes. Try `summary(phdata(srdta))`.

With R, it is also easy to explore the data graphically. For example, the histogram for **qt1** may be generated by



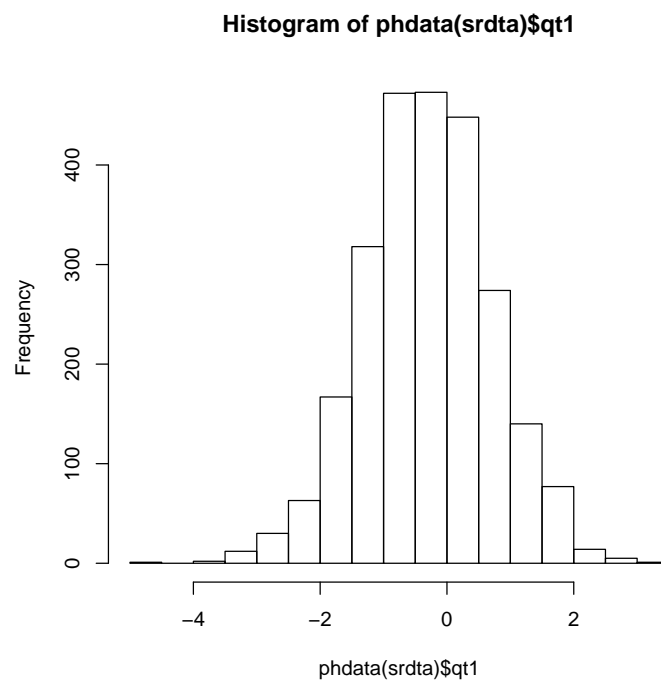


Figure 2.1: Histogram of qt1

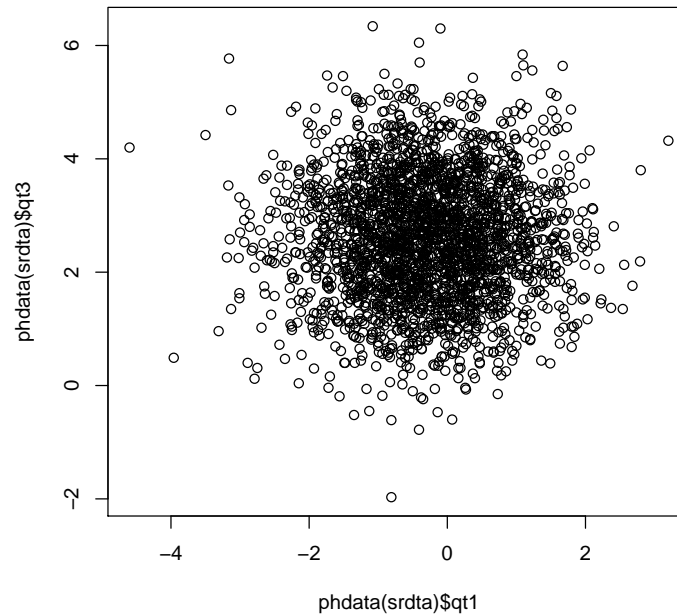


Figure 2.2: Scatter-plot of qt1 against qt3

```
> hist(phdata(srdta)$qt1)
```

(resulting histogram is shown at figure 2.1)

In similar manner, scatter-plots may be generated. To see relation between qt1 and qt3, you can run

```
> plot(phdata(srdta)$qt1, phdata(srdta)$qt3)
```

(resulting plot is shown at figure 2.2)

The mean, median, minimum and maximum of the distribution of the trait may be found out using functions `mean`, `median`, `min` and `max`, respectively. The variance and standard deviation can be computed with `var` and `sd`.

To compute correlation between two variables (or all variables in a matrix/data frame), use `cor`.

In `GenABEL`-package, there is a special function designed to facilitate phenotypic quality control. This function takes names of variables and a data frame as an input, and returns summary statistics, list of outliers (using False Discovery Rate) and graphs.

For example, to do QC of sex, age and qt3, try

```
> check.trait(c("sex", "age", "qt3"), phdata(srdta))
```

```
-----
Trait sex has 2500 measurements
Missing: 0 ( 0 %)
```

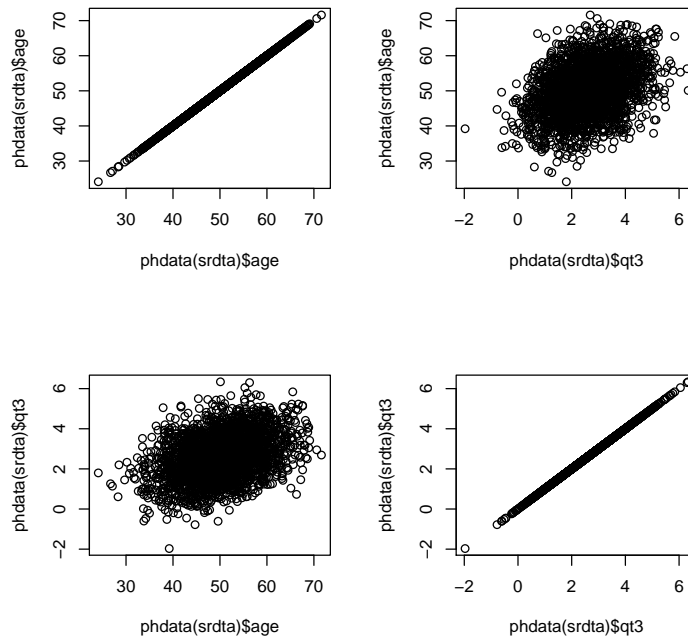


Figure 2.3: Quality control graph for sex, age, qt3

```

Mean = 0.51 ; s.d. = 0.5
NO outliers discovered for trait sex
-----
Trait age has 2500 measurements
Missing: 0 ( 0 %)
Mean = 50.0378 ; s.d. = 7.060125
NO outliers discovered for trait age
-----
Trait qt3 has 2489 measurements
Missing: 11 ( 0.44 %)
Mean = 2.60859 ; s.d. = 1.101154
NO outliers discovered for trait qt3

```

The corresponding graph is depicted at figure ??.

Before you start with the exercise: if a function returns unexpected results, and you are confident that syntax was right, checking help page is always a good idea!

#### Exercise 8. Exploring phenotypic part of srdta

Explore `phdata` part of `srdta` object

1. How many people has age over 65 years?
2. What is the sex distribution (proportion of males) in the people over 65 years old?
3. What is the mean, median, minimum and maximum age in the sample?
4. Compare the distribution of `qt3` in people younger and older than 65 years. Use function `sd(A)` to get standard deviation of `A`
5. Produce distributions of different traits. Do you see something special?
6. What is correlation between `qt3` and `age`?

## 2.4 Regression analysis

While contingency tables, bi-plots and correlation are powerful tools to analyse relations between pairs of variable, a more general framework allowing investigation of relation of an outcome to multiple predictors is regression. In R, function `lm` implements linear regression modelling, and function `glm` implements generalised linear regression. In this section, we will use these two functions to analyse quantitative and binary outcomes.

You can do linear regression to check if trait `qt2` has relation with `sex` and `age` by

```
> a <- lm(phdata(srdta)$qt2 ~ phdata(srdta)$age + phdata(srdta)$sex)
```

The results of analysis are stored in object `'a'`, which has class `'lm'` and contains many sub-objects:

```
> class(a)

[1] "lm"

> names(a)

[1] "coefficients" "residuals"      "effects"        "rank"
[5] "fitted.values" "assign"         "qr"            "df.residual"
[9] "xlevels"      "call"          "terms"         "model"
```

At this moment you do not need to understand all these sub-objects; the meaningful summary of analysis is produced with

```
> summary(a)

Call:
lm(formula = phdata(srdta)$qt2 ~ phdata(srdta)$age + phdata(srdta)$sex)

Residuals:
    Min       1Q   Median       3Q      Max
-5.65  -1.80  -1.03  -0.31  883.08

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
                
```

```
(Intercept)      -1.55892    4.41667  -0.353    0.724
phdata(srdta)$age  0.14022    0.08668   1.618    0.106
phdata(srdta)$sex  1.30377    1.22393   1.065    0.287
```

```
Residual standard error: 30.59 on 2497 degrees of freedom
Multiple R-squared:  0.001518,    Adjusted R-squared:  0.0007181
F-statistic: 1.898 on 2 and 2497 DF,  p-value: 0.1501
```

You can see that `qt2` is not associated with age or sex.

As before, to make easy access to your data (basically, to avoid typing `phdata(srdta)` before every trait name, you may attach the data to the search path:

```
> attach(phdata(srdta))
```

Then, the above expression to run linear regression analysis simplifies to:

```
> summary(lm(qt2 ~ age + sex))
```

Call:

```
lm(formula = qt2 ~ age + sex)
```

Residuals:

```
      Min       1Q   Median       3Q      Max
-5.65  -1.80  -1.03  -0.31  883.08
```

Coefficients:

```
              Estimate Std. Error t value Pr(>|t|)
(Intercept) -1.55892    4.41667  -0.353    0.724
age          0.14022    0.08668   1.618    0.106
sex          1.30377    1.22393   1.065    0.287
```

```
Residual standard error: 30.59 on 2497 degrees of freedom
Multiple R-squared:  0.001518,    Adjusted R-squared:  0.0007181
F-statistic: 1.898 on 2 and 2497 DF,  p-value: 0.1501
```

with the same results.

Analysis of binary outcomes may be performed using `glm` function, using *binomial family* for the error distribution and the link function. For example, to figure out if your binary trait (`bt`) is associated with sex and age, you need to tell that this is binary trait:

```
> a <- glm(bt ~ age + sex, family="binomial")
> summary(a)
```

Call:

```
glm(formula = bt ~ age + sex, family = "binomial")
```

Deviance Residuals:

```
      Min       1Q   Median       3Q      Max
-1.992  -1.091  -0.444   1.094   1.917
```

Coefficients:

	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	-4.639958	0.330519	-14.038	< 2e-16 ***
age	0.088860	0.006463	13.749	< 2e-16 ***
sex	0.379593	0.084138	4.512	6.44e-06 ***

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 3450.5 on 2488 degrees of freedom  
 Residual deviance: 3216.5 on 2486 degrees of freedom  
 (11 observations deleted due to missingness)  
 AIC: 3222.5

Number of Fisher Scoring iterations: 4

There is strong association between `bt` and sex and age. If you want to characterise the strength of association to a binary trait with Odds Ratios, take the exponents of the regression coefficient. For example, the odds ratio associated with male is

```
> exp(0.3796)
```

```
[1] 1.4617
```

## 2.5 Answers to exercises

### Answer of Exercise 1. Explore help for Wilcoxon test

By default (if 'exact' is not specified), an exact p-value is computed if the samples contain less than 50 finite values and there are no ties. Otherwise, a normal approximation is used.

### Answer of Exercise 2. Finding functions and help pages

Try `help.search("Fisher")` and `help.search("Student t-test")`. You will find that the corresponding functions are `fisher.test` `t.test`.

### Answer of Exercise 3. Exploring `srdta`

For the first person id is "p1" and sex code is 1 (1=male, 0=female)

```
> idnames(srdta)[1]
```

```
[1] "p1"
```

```
> male(srdta)[1]
```

```
p1
1
```

For the 22nd person id is "p22" and sex code is 1:

```
> idnames(srdta)[22]
```

```
[1] "p22"
```

```
> male(srdta)[22]
```

```
p22
```

```
1
```

Among first 100 subjects, there are 53 males:

```
> sum(male(srdta)[1:100])
```

```
[1] 53
```

Among 4th hundred subjects there are 45 females:

```
> 100-sum(male(srdta)[301:400])
```

```
[1] 45
```

Male proportion among first 1000 people is

```
> mean(male(srdta)[1:1000])
```

```
[1] 0.508
```

Female proportion among second 1000 people is

```
> 1 - mean(male(srdta)[1001:2000])
```

```
[1] 0.476
```

Name, chromosome and map position of the 33rd marker are:

```
> snpnames(srdta)[33]
```

```
[1] "rs422"
```

```
> chromosome(srdta)[33]
```

```
rs422
```

```
"1"
```

```
> map(srdta)[33]
```

```
rs422
```

```
105500
```

The map positions for and distance between markers 25 and 26 are:

```
> pos25 <- map(srdta)[25]
```

```
> pos25
```

```
rs365
```

```
91250
```

```
> pos26 <- map(srdta)[26]
> pos26
```

```
rs372
92750
```

```
> pos26-pos25
```

```
rs372
1500
```

#### Answer of Exercise 4. Exploring assoc

Here is an automatic script which explores the classes of variables in `assoc`:

```
> for (i in names(assoc)) {
+   cat("Variable '", i, "' has class '", class(assoc[,i]), "'\n", sep="")
+ }
```

```
Variable 'subj' has class 'integer'
Variable 'sex' has class 'numeric'
Variable 'aff' has class 'numeric'
Variable 'qt' has class 'numeric'
Variable 'snp4' has class 'character'
Variable 'snp5' has class 'character'
Variable 'snp6' has class 'character'
```

#### Answer of Exercise 5. Explore phenotypic part of srdta

Load the data and look at the few first rows of the phenotypic data frame:

```
> data(srdta)
> phdata(srdta)[1:5,]

  id sex  age  qt1  qt2 qt3 bt
p1 p1   1 43.4 -0.58  4.46 1.43 0
p2 p2   1 48.2  0.80  6.32 3.90 1
p3 p3   0 37.9 -0.52  3.26 5.05 1
p4 p4   1 53.8 -1.55 888.00 3.76 1
p5 p5   1 47.5  0.25  5.70 2.89 1
```

Value of the 4th variable of person 75:

```
> phdata(srdta)[75,4]
```

```
[1] -0.04
```

Value for the variable 1 is

```
> phdata(srdta)[75,1]
```

```
[1] "p75"
```

Also, if we check first 10 elements we see



```
> phdata(srdta)[1:10,1]
```

```
[1] "p1" "p2" "p3" "p4" "p5" "p6" "p7" "p8" "p9" "p10"
```

This is personal ID.

The sum for variable 2 is

```
> sum(phdata(srdta)[,2])
```

```
[1] 1275
```

This is sex variable – so there are 1275 males in the data set.

### Answer of Exercise 6. Explore assoc

The number of affected (coded with '1') and unaffected ('0') is

```
> table(aff)
```

```
aff
 0  1
190 55
```

The proportion of unaffected and affected is

```
> prop.table(table(aff))
```

```
aff
      0      1
0.7755102 0.2244898
```

Distribution of the 'snp4' is

```
> t <- table(snp4)
> t
```

```
snp4
A/A A/B B/B
109 105 29
```

```
> prop.table(t)
```

```
snp4
  A/A    A/B    B/B
0.4485597 0.4320988 0.1193416
```

### Answer of Exercise 7. Explore phenotypes in srdta

Number of people:

```
> nids(srdta)
```

```
[1] 2500
```

Number of variables:

```
> length(names(phdata(srdta)))
```

```
[1] 7
```

The same – dimensions of phenotypic data frame:

```
> dim(phdata(srdta))
```

```
[1] 2500    7
```

Class of variables in phenotypic data frame:

```
> for (i in names(phdata(srdta))) {
+   cat("class of variable '", i, "' is '", class(phdata(srdta)[,i]), "'\n", s
+ }
```

```
class of variable 'id' is 'character'
class of variable 'sex' is 'integer'
class of variable 'age' is 'numeric'
class of variable 'qt1' is 'numeric'
class of variable 'qt2' is 'numeric'
class of variable 'qt3' is 'numeric'
class of variable 'bt' is 'integer'
```

### Answer of Exercise 8. Exploring phenotypic part of srdta

To obtain the number of people with age >65 y.o., you can use any of the following

```
> sum(phdata(srdta)$age>65)
```

```
[1] 48
```

```
> vec <- which(phdata(srdta)$age>65)
```

```
> length(vec)
```

```
[1] 48
```

To get sex of these people use any of:

```
> sx65 <- phdata(srdta)$sex[phdata(srdta)$age>65]
```

```
> sx65
```

```
[1] 1 1 0 0 0 0 1 1 1 1 0 0 1 1 0 1 1 1 0 0 0 0 1 1 1 0 1 1 1 1 0 1 1 1 0 0 0 0
[39] 1 0 1 0 0 0 0 1 1 1
```

```
> sx65 <- phdata(srdta)$sex[vec]
```

```
> sx65
```

```
[1] 1 1 0 0 0 0 1 1 1 1 0 0 1 1 0 1 1 1 0 0 0 0 1 1 1 0 1 1 1 1 0 1 1 1 0 0 0 0
[39] 1 0 1 0 0 0 0 1 1 1
```

Thus, number of males is:

```
> sum(sx65)
```

```
[1] 26
```

To conclude, the proportion of male is 0.541666666666667.

Distribution of `qt3` in people younger and older than 65 are:

```
> summary(phdata(srdta)$qt3[vec])
```

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
0.730	2.690	3.480	3.499	4.265	5.840

```
> sd(phdata(srdta)$qt3[vec],na.rm=TRUE)
```

```
[1] 1.128701
```

```
> young <- which(phdata(srdta)$age<65)
```

```
> summary(phdata(srdta)$qt3[young])
```

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.	NA's
-1.97	1.83	2.58	2.59	3.35	6.34	11

```
> sd(phdata(srdta)$qt3[young],na.rm=TRUE)
```

```
[1] 1.093374
```

Mean, median, min and max of age:

```
> summary(phdata(srdta)$age)
```

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
24.10	45.10	50.00	50.04	54.80	71.60

The histogram for `qt2` looks strange (you can generate that using `hist(phdata(srdta)$qt2)`): it seems there are few very strong outliers. You can also see that with `summary`:

```
> summary(phdata(srdta)$qt2)
```

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
0.000	4.220	5.045	6.122	5.910	888.000



## Chapter 3

# Introduction to genetic association analysis in R

When analyzing several (dozens of) SNPs, facilities of base R are sufficient and efficient for data storage and analysis. Few specific test, such as these of Hardy-Weinberg Equilibrium (HWE) and Linkage Disequilibrium (LD), are implemented in different libraries, e.g. **genetics** and **GenABEL-package**.

In this section, we will describe library **genetics** and will make use of it to guide you through simple genetic analysis exercise using a small example data set. In the last part, you will investigate a bigger data set as based on the knowledge obtained in the first part, and will answer the questions.

### 3.1 Characterisation of genetic data

### 3.2 Exploring genetic data with library **genetics**

Library **genetics** was written by Gregory R. Warnes to facilitate analysis of genetic data in R. This library

- Implements genetic analysis tests, such as test for Hardy-Weinberg equilibrium and Linkage disequilibrium.
- Implements new data classes, such as **genotype**, **haplotype** and **LD.data.frame**.
- Modifies default R functions, such as **summary** and **plot** to correctly analyse and present these new classes.
- Facilitates export of the data from R to the formats supported by such genetic analysis packages as GenePop and QTDT.

Start R by double-click on the file **ge03d1p1.RData**. Load library **genetics**, which we will need for testing HWE and computations of LD by

```
> library(genetics)
```

NOTE: THIS PACKAGE IS NOW OBSOLETE.

The R-Genetics project has developed an set of enhanced genetics packages to replace 'genetics'. Please visit the project homepage at <http://rgenetics.org> for information.

The file you have loaded contains single data frame `assocg`. Let us briefly explore it:

```
> class(assocg)

[1] "data.frame"

> names(assocg)

[1] "subj" "sex"  "aff"  "qt"   "snp4" "snp5" "snp6"

> dim(assocg)

[1] 250  7
```

You can see that `assocg` looks remarkably similar to the previously explored data frame `assoc` (section 2.2, page 20). Indeed, they are almost equivalent. Let us present the data for the subjects 5 to 15 and compare this output to that presented on page 23:

```
> assocg[5:15,]

      subj sex aff      qt snp4 snp5 snp6
1533 1533  0  0  0.1009220 A/B  B/A  B/A
2466 2466  1  0 -0.1724321 A/B  A/A  A/A
2425 2425  0  0 -0.3378473 B/B  A/A  A/A
1068 1068  0  0 -1.7112925 A/A  B/B <NA>
198   198  1  0 -0.4815822 A/B  B/A  B/A
1496 1496  1  0  1.2281232 A/A  B/B  B/B
909   909  0  0  0.5993945 A/B  B/A  B/A
1213 1213  0  0  1.9792190 A/A  B/B  B/B
181   181  1  0  1.5435921 A/A  B/B  B/B
1783 1783  0  0 -1.6242738 A/B  B/A  B/A
1914 1914  0  0 -0.5160331 A/A  B/B  B/B
```

The data are identical. However, the SNP data presented in the new data frame have special class `genotype`, as implemented in `genetics` library:

```
> class(assocg$snp4)

[1] "genotype" "factor"
```

Previously, the SNP genotypes were coded as characters. This new way of presentation allows library `genetics` to recognise the SNP data as genetic and analyse them accordingly.

Let us attach the `assocg` data frame and explore what data analysis advantages are achieved by application of library `genetics`.

```
> attach(assocg)
```

As we noted in section 2.2, testing Hardy-Weinberg equilibrium, testing allelic effects, and even computation of allelic frequency is not so straightforward in base R. These tests, are, however, easy with library **genetics**. To see the allelic frequencies and other summary statistics for a SNP, you can use

```
> summary(snp4)
```

Number of samples typed: 243 (97.2%)

Allele Frequency: (2 alleles)

	Count	Proportion
A	323	0.66
B	163	0.34
NA	14	NA

Genotype Frequency:

	Count	Proportion
B/B	29	0.12
A/B	105	0.43
A/A	109	0.45
NA	7	NA

Heterozygosity (Hu) = 0.4467269  
Poly. Inf. Content = 0.3464355

To check these characteristics in controls and cases separately, you can use

```
> summary(snp4[aff==0])
```

Number of samples typed: 190 (97.9%)

Allele Frequency: (2 alleles)

	Count	Proportion
A	255	0.67
B	125	0.33
NA	8	NA

Genotype Frequency:

	Count	Proportion
B/B	22	0.12
A/B	81	0.43
A/A	87	0.46
NA	4	NA

Heterozygosity (Hu) = 0.4426469  
Poly. Inf. Content = 0.3440288

```
> summary(snp4[aff==1])
```

Number of samples typed: 53 (94.6%)

Allele Frequency: (2 alleles)

	Count	Proportion
A	68	0.64
B	38	0.36
NA	6	NA

Genotype Frequency:

	Count	Proportion
B/B	7	0.13
A/B	24	0.45
A/A	22	0.42
NA	3	NA

Heterozygosity (Hu) = 0.4643306

Poly. Inf. Content = 0.3541731

Let us check if HWE holds for the SNPs described in this data frame. We can do exact test for HWE by

```
> HWE.exact(snp4)
```

Exact Test for Hardy-Weinberg Equilibrium

data: snp4

N11 = 109, N12 = 105, N22 = 29, N1 = 323, N2 = 163, p-value = 0.666

If you want to check HWE using controls only, you can do it by

```
> HWE.exact(snp4[aff==0])
```

Exact Test for Hardy-Weinberg Equilibrium

data: snp4[aff == 0]

N11 = 87, N12 = 81, N22 = 22, N1 = 255, N2 = 125, p-value = 0.6244

Let us check if there is LD between snp4 and snp5:

```
> LD(snp4,snp5)
```

Pairwise LD

	D	D'	Corr
Estimates:	0.2009042	0.9997352	0.8683117

	X <sup>2</sup>	P-value	N
LD Test:	354.3636	0	235

The output shows results of the test for significance of LD, and estimates of the magnitude of LD ( $D'$  and correlation,  $r$ ). To obtain  $r^2$ , you can either square the correlation manually



```
> 0.8683117*0.8683117
```

```
[1] 0.7539652
```

or simply ask `LD()` to report it by

```
> LD(snp4, snp5)$"R^2"
```

```
[1] 0.7539652
```

The latter command is possible because the `LD()` function actually computes more things than it reports. This is quite common for R functions. You can apply `names()` function to the analysis objects to see (at least part of) what was actually computed. Try

```
> ld45 <- LD(snp4, snp5)
```

and check what are the sub-objects contained in this analysis object

```
> names(ld45)
```

```
[1] "call"      "D"         "D'"        "r"         "R^2"       "n"         "X^2"
[8] "P-value"
```

Any of these variables can be accessed through `object$var` syntax, e.g. to check  $D'$  we can use

```
> ld45$"D'"
```

```
[1] 0.9997352
```

To check LD for more than two SNPs, we can compute an LD analysis object by

```
> ldall <- LD(data.frame(snp4, snp5, snp6))
```

and later check

```
> ldall$"P-value"
```

```
      snp4 snp5 snp6
snp4   NA    0    0
snp5   NA   NA    0
snp6   NA   NA   NA
```

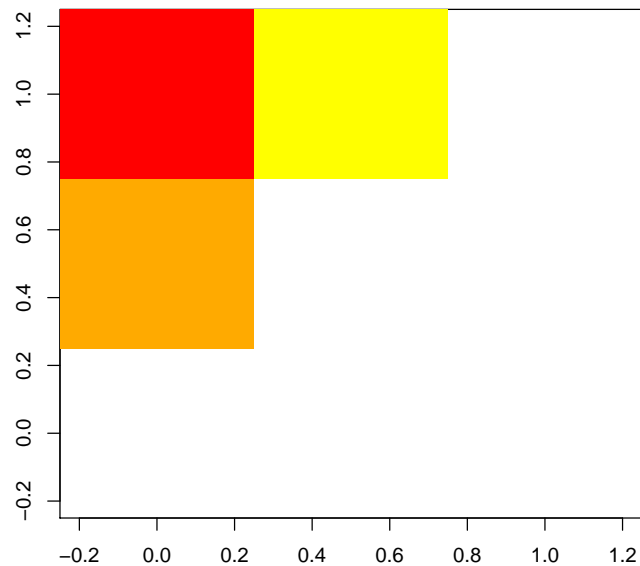
to see significance,

```
> ldall$"D'"
```

```
      snp4      snp5      snp6
snp4   NA 0.9997352 0.8039577
snp5   NA      NA 0.9997231
snp6   NA      NA      NA
```

for  $D'$  and

```
> ldall$"R^2"
```

Figure 3.1:  $r^2$  plot for snp4, snp5 and snp6

	snp4	snp5	snp6
snp4	NA	0.7539652	0.5886602
snp5	NA	NA	0.8278328
snp6	NA	NA	NA

for  $r^2$ .

You can also present e.g.  $r^2$  matrix as a plot by

```
> image(ldall$"R^2")
```

A more neat way to present it requires specification of the set of threshold (break points) and colors to be used (you do not need to try this example if you do not want):

```
> image(ldall$"R^2", breaks=c(0.5, 0.6, 0.7, 0.8, 0.9, 1), col=heat.colors(5))
```

Resulting plot is shown at figure 3.1.

**For any R command, you can get help by typing `help(command)`. Try `help(image)` if you are interested to understand what are "breaks" and "col"; or try `help(heat.colors)` to figure this color schema out.**

Similar to our HWE checks, we may want to compute (and compare) LD in cases and controls separately:

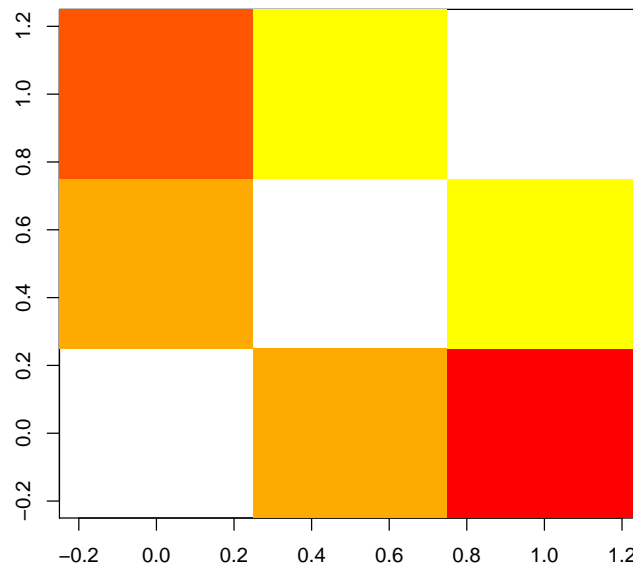


Figure 3.2:  $r^2$  plot for snp4, snp5 and snp6. Above diagonal: LD in cases; below: controls

```
> ldcases <- LD(data.frame(snp4,snp5,snp6)[aff==1,])
> ldcases$"R^2"
```

	snp4	snp5	snp6
snp4	NA	0.7615923	0.6891558
snp5	NA	NA	0.8943495
snp6	NA	NA	NA

```
> ldcontr <- LD(data.frame(snp4,snp5,snp6)[aff==0,])
> ldcontr$"R^2"
```

	snp4	snp5	snp6
snp4	NA	0.7512458	0.5616395
snp5	NA	NA	0.8075894
snp6	NA	NA	NA

and even present it results for cases and controls on the same graph (you do not need to produce this graph, which is presented at the figure 3.2):

```
> image(ldcases$"R^2",breaks=c(0.5,0.6,0.7,0.8,0.9,1),col=heat.colors(5))
> image(t(ldcontr$"R^2"),breaks=c(0.5,0.6,0.7,0.8,0.9,1),col=heat.colors(5),add=T)
```

### 3.3 Genetic association analysis

### 3.4 Example association analysis

Now, after we have described genetic and phenotypic data separately, we are ready to test association between these two. In previous sections, we showed that association between a binary trait and genotype may be analysed using contingency tables (functions `table`, `prop.table`, `fisher.test`, etc.). The association between a quantitative trait and genotype may be done using correlations, T-test, etc.

However, a more flexible analysis is possible when using regression modelling. First, we will investigate relation between the quantitative trait `qt` and the SNPs by using linear regression

```
> mg <- lm(qt ~ snp4)
```

The `lm` command fits linear regression model to the data and returns an analysis object. The summary of analysis may be generated with

```
> summary(mg)
```

Call:

```
lm(formula = qt ~ snp4)
```

Residuals:

	Min	1Q	Median	3Q	Max
	-2.63700	-0.62291	-0.01225	0.58922	3.05561

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.081114	0.092517	-0.877	0.382
snp4A/B	-0.108366	0.132079	-0.820	0.413
snp4B/B	-0.006041	0.201820	-0.030	0.976

Residual standard error: 0.9659 on 240 degrees of freedom

(7 observations deleted due to missingness)

Multiple R-squared: 0.003049, Adjusted R-squared: -0.005259

F-statistic: 0.367 on 2 and 240 DF, p-value: 0.6932

From the summary output, it is clear that the model assumes arbitrary (estimated) effects of the genotypes AA, AB and BB. Neither effect of AB nor BB is significant in this case. The global test on two degrees of freedom (bottom of the output) is also not significant.

If you want to include some covariate into your model, e.g. `sex`, you can easily do that by adding the term to the formula:

```
> summary(lm(qt ~ sex + snp4))
```

Call:

```
lm(formula = qt ~ sex + snp4)
```

Residuals:

	Min	1Q	Median	3Q	Max
	-2.66442	-0.62417	-0.00875	0.59705	3.08086

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.110298	0.115260	-0.957	0.340
sex	0.053018	0.124493	0.426	0.671
snp4A/B	-0.104429	0.132628	-0.787	0.432
snp4B/B	-0.002452	0.202340	-0.012	0.990

Residual standard error: 0.9676 on 239 degrees of freedom

(7 observations deleted due to missingness)

Multiple R-squared: 0.003805, Adjusted R-squared: -0.0087

F-statistic: 0.3043 on 3 and 239 DF, p-value: 0.8223

You can also allow for interaction by using the "\*" operator

```
> summary(lm(qt~sex*snp4))
```

Call:

```
lm(formula = qt ~ sex * snp4)
```

Residuals:

	Min	1Q	Median	3Q	Max
	-2.57049	-0.64596	-0.00264	0.61094	3.01970

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.20579	0.13834	-1.487	0.138
sex	0.22649	0.18647	1.215	0.226
snp4A/B	0.05222	0.19024	0.274	0.784
snp4B/B	0.18071	0.28576	0.632	0.528
sex:snp4A/B	-0.30191	0.26566	-1.136	0.257
sex:snp4B/B	-0.35508	0.40531	-0.876	0.382

Residual standard error: 0.9684 on 237 degrees of freedom

(7 observations deleted due to missingness)

Multiple R-squared: 0.01041, Adjusted R-squared: -0.01047

F-statistic: 0.4984 on 5 and 237 DF, p-value: 0.7773

Note that both main effects of sex and snp4, and also effects of interaction are estimated in this model.

Of interest in genetic studies may be three other models: additive, dominant and recessive.

The additive model assumes that the difference between mean trait's values between 'AA' and 'BB' is twice the difference between 'AA' and 'BB', that is the mean value of the trait for heterozygous genotypes is right in between the two homozygotes. To test additive model, we first need to recode the predictor (genotype) as a numeric factor to be used as covariate. This can be easily done with function `as.numeric`:

```
> add4 <- as.numeric(snp4)-1
```

We can check if recoding was done correctly by producing the table

```
> table(snp4, add4)
```

```
      add4
snp4    0    1    2
A/A  109    0    0
A/B    0 105    0
B/B    0    0   29
```

Now to test the additive model run

```
> summary(lm(qt~add4))
```

Call:

```
lm(formula = qt ~ add4)
```

Residuals:

```
      Min       1Q   Median       3Q      Max
-2.54813 -0.62104 -0.02754  0.60584  3.00652
```

Coefficients:

```
              Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.10476     0.08710  -1.203   0.230
add4         -0.03563     0.09133  -0.390   0.697
```

Residual standard error: 0.9651 on 241 degrees of freedom

(7 observations deleted due to missingness)

Multiple R-squared: 0.0006313, Adjusted R-squared: -0.003516

F-statistic: 0.1522 on 1 and 241 DF, p-value: 0.6968

The model assuming dominant action of the 'A' allele means that the means of genotypes 'AA' and 'AB' are the same. This is equivalent to the model of recessive action of 'B' allele. To code SNP4 according to this model, we can use function `replace`:

```
> dom4 <- add4
> dom4[dom4==2] <- 1
> table(snp4, dom4)
```

```
      dom4
snp4    0    1
A/A  109    0
A/B    0 105
B/B    0   29
```

To test association with a binary outcome, we will use function `glm` with binomial family:

```
> summary(glm(aff~snp4, family="binomial"))
```

```

Call:
glm(formula = aff ~ snp4, family = "binomial")

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.7433 -0.7204 -0.6715 -0.6715  1.7890

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept) -1.3749     0.2386  -5.761 8.35e-09 ***
snp4A/B       0.1585     0.3331   0.476  0.634
snp4B/B       0.2297     0.4952   0.464  0.643
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 254.91  on 242  degrees of freedom
Residual deviance: 254.58  on 240  degrees of freedom
(7 observations deleted due to missingness)
AIC: 260.58

Number of Fisher Scoring iterations: 4

To make a test of global significance of the SNP effect, you can use
> anova(glm(aff~snp4,family="binomial"),test="Chisq")

Analysis of Deviance Table

Model: binomial, link: logit

Response: aff

Terms added sequentially (first to last)

      Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                    242      254.91
snp4  2  0.32894      240      254.58  0.8483

In the manner similar to that described for quantitative traits, additive and dominance/recessive models can be tested by proper coding of the genotypic variable, e.g. to test the additive model, use

> summary(glm(aff~as.numeric(snp4),family="binomial"))

Call:
glm(formula = aff ~ as.numeric(snp4), family = "binomial")

Deviance Residuals:

```

Min	1Q	Median	3Q	Max
-0.7549	-0.7139	-0.6747	-0.6747	1.7842

Coefficients:

	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	-1.4913	0.4164	-3.581	0.000342 ***
as.numeric(snp4)	0.1272	0.2268	0.561	0.574994

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 254.91 on 242 degrees of freedom  
 Residual deviance: 254.60 on 241 degrees of freedom  
 (7 observations deleted due to missingness)  
 AIC: 258.6

Number of Fisher Scoring iterations: 4

Now you have learned all commands necessary to answer the questions of the next section.

Exit R by typing `q()` command (do not save image) and proceed to the self exercise.

### 3.5 Exercise: Exploring genetic data using library genetics

Start R by double-click over the file `ge03d1p2.RData` (Windows) or by changing to the directory containing the data, starting R and loading the data set with `load("ge03d1p2.RData")` (Linux). Explore the data frame present and answer the questions.

**Ex. 1** — How many SNPs are described in this data frame?

**Ex. 2** — What is the frequency (proportion) of `snp1` allele 'A'?

**Ex. 3** — What is its frequency of 'A' in affected (`aff==1`)?

**Ex. 4** — How many cases and controls are present in the data set?

**Ex. 5** — If all subjects are used to test HWE, are there any SNPs out of HWE at nominal  $P \leq 0.05$ ? Which ones?

**Ex. 6** — If only controls are used to test the SNPs which are out of HWE in total sample, are these still out of HWE?

**Ex. 7** — Which SNP pairs are in strong LD ( $r^2 \geq 0.8$ )?

**Ex. 8** — For SNPs in strong LD, what is  $r^2$  for separate samples of cases and controls?



**Ex. 9** — Is there significant association between affection status and sex? What is  $P$ -value for association?

**Ex. 10** — Is association between the disease and `qt` significant?

**Ex. 11** — Which SNPs are significantly associated with affection status at nominal  $p$ -value  $\leq 0.05$ ? Use general genotypic (2 d.f.) model.

**Ex. 12** — Test association between `aff` and `snp5` and `snp10`, allowing for the SNPs interaction effect. Use arbitrary (not an additive) model. Do you observe significant interaction? How can you describe the model of concert action of `snp5` and `snp10`?

**Ex. 13** — Test for association between the quantitative trait `qt` and SNPs 1-10 using additive model. Which SNPs are associated at nominal  $P \leq 0.05$ ?

**Ex. 14** — **OPTIONAL, difficulty is medium, but may be time-consuming.**

If you adjust the analysis under additive model for sex, how do the findings change? Before doing the exercise, please check the answer to **previous** exercise – it shows a quick way to do testing for all 10 SNPs.

**Ex. 15** — Which SNPs are associated with the quantitative trait `qt` at nominal  $P \leq 0.05$  when general genotypic (2 d.f. test) model is used?

**Ex. 16** — **ADVANCED:** How can you describe the model of action of the significant SNPs? Test if the data are compatible with additive/dominant/recessive model.

## 3.6 Answers to exercises

**Answer (Ex. 1)** — The answer is 10 snps:

```
> attach(popdat)
> names(popdat)

[1] "subj"  "sex"   "aff"   "qt"    "snp1"  "snp2"  "snp3"  "snp4"  "snp5"
[10] "snp6"  "snp7"  "snp8"  "snp9"  "snp10"
```

**Answer (Ex. 2)** — The frequency of 'A' in all subjects is 0.73:

```
> summary(snp1)

Number of samples typed: 2374 (95%)

Allele Frequency: (2 alleles)
  Count Proportion
A   3462      0.73
B   1286      0.27
NA    252       NA
```

```

Genotype Frequency:
      Count Proportion
B/B    199         0.08
A/B    888         0.37
A/A   1287         0.54
NA     126          NA

```

```

Heterozygosity (Hu) = 0.3950646
Poly. Inf. Content  = 0.3169762

```

**Answer (Ex. 3)** — The frequency of A in affected subjects is 0.7:

```

> summary(snp1[aff==1])
Number of samples typed: 519 (94.5%)

```

```

Allele Frequency: (2 alleles)
      Count Proportion
A       729         0.7
B       309         0.3
NA       60          NA

```

```

Genotype Frequency:
      Count Proportion
B/B     48         0.09
A/B    213         0.41
A/A    258         0.50
NA     30          NA

```

```

Heterozygosity (Hu) = 0.4185428
Poly. Inf. Content  = 0.3307192

```

**Answer (Ex. 4)** — There are 549 cases and 1951 controls:

```

> table(aff)
aff
  0    1
1951 549

```

**Answer (Ex. 5)** — Only SNP 1 is out of HWE in the total sample. Here is a script testing all SNPs (no need to reproduce that, just check the results):

```

> for (i in 1:10) {
+   snpname <- paste("snp",i,sep="")
+   cat("HWE P-value for SNP",snpname,"is",HWE.exact(get(snpname))$p.value,"\n")
+ }

HWE P-value for SNP snp1 is 0.01083499
HWE P-value for SNP snp2 is 1

```

```

HWE P-value for SNP snp3 is 0.4197772
HWE P-value for SNP snp4 is 0.8960298
HWE P-value for SNP snp5 is 0.2960967
HWE P-value for SNP snp6 is 0.5207056
HWE P-value for SNP snp7 is 0.6284575
HWE P-value for SNP snp8 is 0.1309458
HWE P-value for SNP snp9 is 0.4457363
HWE P-value for SNP snp10 is 0.7897327

```

**Answer (Ex. 6)** — Yes, SNP one is out of HWE also in controls:

```

> HWE.exact(snp1[aff==0])
      Exact Test for Hardy-Weinberg Equilibrium

data:  snp1[aff == 0]
N11 = 1029, N12 = 675, N22 = 151, N1 = 2733, N2 = 977, p-value =
0.008393

```

**Answer (Ex. 7)** — SNP pairs 4-5 and 5-6 have  $r^2 \geq 0.8$ :

```

> LD(popdat[,5:14])$"R^2"
      snp1  snp2  snp3  snp4  snp5  snp6  snp7  snp8  snp9  snp10
snp1    NA 0.016 0.235 0.206 0.258 0.227 0.152 0.117 0.090 0.000
snp2    NA    NA 0.004 0.004 0.005 0.004 0.000 0.000 0.000 0.000
snp3    NA    NA    NA 0.602 0.457 0.346 0.641 0.031 0.042 0.001
snp4    NA    NA    NA    NA 0.803 0.650 0.729 0.027 0.037 0.002
snp5    NA    NA    NA    NA    NA 0.874 0.586 0.034 0.046 0.002
snp6    NA    NA    NA    NA    NA    NA 0.670 0.030 0.040 0.002
snp7    NA    NA    NA    NA    NA    NA    NA 0.020 0.027 0.003
snp8    NA    NA    NA    NA    NA    NA    NA    NA 0.002 0.000
snp9    NA    NA    NA    NA    NA    NA    NA    NA    NA 0.001
snp10   NA    NA    NA    NA    NA    NA    NA    NA    NA    NA

```

**Answer (Ex. 8)** — For controls,

```

> #LD(popdat[aff==0,8:10])$"R^2"
> LD(data.frame(snp4,snp5,snp6)[aff==0,])$"R^2"
      snp4      snp5      snp6
snp4    NA 0.806591 0.6419715
snp5    NA      NA 0.8661005
snp6    NA      NA      NA

```

For cases,

```

> #LD(popdat[aff==1,8:10])$"R^2"
> LD(data.frame(snp4,snp5,snp6)[aff==1,])$"R^2"
      snp4      snp5      snp6
snp4    NA 0.7951475 0.6773275

```

```
snp5  NA      NA 0.9083237
snp6  NA      NA      NA
```

Note that the fact that LD is higher in cases may mean nothing because the estimates of LD are biased upwards with smaller sample sizes. For example in a small sample (5 people) of controls we expect even higher LD because of strong upward bias:

```
> LD(popdat[which(aff==0)[1:5],8:10])$"R^2"

      snp4      snp5      snp6
snp4  NA 0.9995876 0.9995876
snp5  NA      NA 0.9995876
snp6  NA      NA      NA
```

More elaborate methods, such as that by ZAYKIN *et al.* (2006), are required to contrast LD between sample of unequal size.

**Answer (Ex. 9)** — There is no significant association:

```
> t <- table(aff,sex)
> t

      sex
aff  0   1
  0 973 978
  1 260 289
> fisher.test(t)

      Fisher's Exact Test for Count Data

data:  t
p-value = 0.3104
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 0.9107753 1.3430565
sample estimates:
odds ratio
 1.105811
> summary(glm(aff~sex,family=binomial()))
Call:
glm(formula = aff ~ sex, family = binomial())

Deviance Residuals:
      Min       1Q   Median       3Q      Max
-0.7196  -0.7196  -0.6882  -0.6882   1.7644

Coefficients:
              Estimate Std. Error z value Pr(>|z|)
(Intercept) -1.31970    0.06981  -18.90  <2e-16 ***
sex           0.10062    0.09673   1.04   0.298
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 2632.0 on 2499 degrees of freedom  
 Residual deviance: 2630.9 on 2498 degrees of freedom  
 AIC: 2634.9

Number of Fisher Scoring iterations: 4

**Answer (Ex. 10)** — There is no significant association:

```
> summary(glm(aff~qt,family=binomial()))
```

Call:

```
glm(formula = aff ~ qt, family = binomial())
```

Deviance Residuals:

Min	1Q	Median	3Q	Max
-0.7326	-0.7079	-0.7012	-0.6905	1.7675

Coefficients:

	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	-1.26769	0.04832	-26.238	<2e-16 ***
qt	-0.02514	0.04862	-0.517	0.605

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 2632.0 on 2499 degrees of freedom  
 Residual deviance: 2631.7 on 2498 degrees of freedom  
 AIC: 2635.7

Number of Fisher Scoring iterations: 4

**Answer (Ex. 11)** — SNPs 5 and 10 are significantly associated:

```
> for (i in 1:10) {
+   snpname <- paste("snp",i,sep="")
+   cat("\nTesting association between aff and SNP",snpname,"\n")
+   print(anova(glm(aff~get(snpname),family=binomial),test="Chisq"))
+   #print(summary(lm(qt~get(snpname)))$coef)
+ }
```

Testing association between aff and SNP snp1 :  
 Analysis of Deviance Table

Model: binomial, link: logit

Response: aff

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			2373	2493.4	
get(snpname)	2	5.4094	2371	2488.0	0.06689 .

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Testing association between aff and SNP snp2 :

Analysis of Deviance Table

Model: binomial, link: logit

Response: aff

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			2373	2485.8	
get(snpname)	1	0.29367	2372	2485.5	0.5879

Testing association between aff and SNP snp3 :

Analysis of Deviance Table

Model: binomial, link: logit

Response: aff

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			2377	2503.0	
get(snpname)	2	2.6087	2375	2500.4	0.2714

Testing association between aff and SNP snp4 :

Analysis of Deviance Table

Model: binomial, link: logit

Response: aff

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			2389	2519.1	

```
get(snpname) 2 5.2755 2387 2513.8 0.07152 .
```

```
---
```

```
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Testing association between aff and SNP snp5 :
```

```
Analysis of Deviance Table
```

```
Model: binomial, link: logit
```

```
Response: aff
```

```
Terms added sequentially (first to last)
```

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			2382	2440.4	
get(snpname)	2	9.2395	2380	2431.2	0.009855 **

```
---
```

```
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Testing association between aff and SNP snp6 :
```

```
Analysis of Deviance Table
```

```
Model: binomial, link: logit
```

```
Response: aff
```

```
Terms added sequentially (first to last)
```

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			2379	2498.9	
get(snpname)	2	1.7969	2377	2497.1	0.4072

```
Testing association between aff and SNP snp7 :
```

```
Analysis of Deviance Table
```

```
Model: binomial, link: logit
```

```
Response: aff
```

```
Terms added sequentially (first to last)
```

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			2367	2487.9	
get(snpname)	2	1.3604	2365	2486.6	0.5065

```
Testing association between aff and SNP snp8 :
```

```
Analysis of Deviance Table
```

Model: binomial, link: logit

Response: aff

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			2370	2489.4	
get(snpname)	2	5.5375	2368	2483.9	0.06274 .

---  
Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Testing association between aff and SNP snp9 :  
Analysis of Deviance Table

Model: binomial, link: logit

Response: aff

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			2360	2476.8	
get(snpname)	2	1.1891	2358	2475.6	0.5518

Testing association between aff and SNP snp10 :  
Analysis of Deviance Table

Model: binomial, link: logit

Response: aff

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			2383	2475.1	
get(snpname)	2	6.7328	2381	2468.4	0.03451 *

---  
Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Answer (Ex. 12)** — It appears that SNP10 genotype is only relevant in these who are homozygous for the low-risk A allele at the SNP5; in such cases SNP 10 allele B is risk increasing. In these homozygous for SNP 5 A, we observe highly significant increase in risk for heterozygotes for SNP10 and increased (though



not significantly) risk for SNP 10 BB:

```
> summary(glm(aff~snp5*snp10,family=binomial()))
```

Call:

```
glm(formula = aff ~ snp5 * snp10, family = binomial())
```

Deviance Residuals:

Min	1Q	Median	3Q	Max
-0.9906	-0.7340	-0.6323	-0.5215	2.0310

Coefficients:

	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	-1.50840	0.08905	-16.938	< 2e-16 ***
snp5A/A	-0.41802	0.19722	-2.120	0.0340 *
snp5B/B	0.33441	0.13360	2.503	0.0123 *
snp10A/B	-0.01403	0.18251	-0.077	0.9387
snp10B/B	-0.14983	0.55277	-0.271	0.7863
snp5A/A:snp10A/B	1.48369	0.32750	4.530	5.89e-06 ***
snp5B/B:snp10A/B	0.12989	0.27441	0.473	0.6360
snp5A/A:snp10B/B	0.82348	0.98963	0.832	0.4053
snp5B/B:snp10B/B	-0.28562	1.23104	-0.232	0.8165

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 2282.4 on 2268 degrees of freedom

Residual deviance: 2242.9 on 2260 degrees of freedom

(231 observations deleted due to missingness)

AIC: 2260.9

Number of Fisher Scoring iterations: 4

**Answer (Ex. 13)** — SNPs 1, 4, 5, 6 and 9 are significantly associated at nominal  $P \leq 0.05$ . Here is a testing script (no need to reproduce that, just check the results):

```
> for (i in 1:10) {
+   snpname <- paste("snp",i,sep="")
+   cat("\nTesting association between qt and SNP",snpname,"\n")
+   testmodel <- lm(qt~as.numeric(get(snpname)))
+   print(summary(testmodel)$coef)
+ }
```

Testing association between qt and SNP snp1 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.11874800	0.05260279	-2.257447	0.024070746
as.numeric(get(snpname))	0.08859657	0.03147693	2.814651	0.004923315

Testing association between qt and SNP snp2 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.09149145	0.1841115	0.4969352	0.6192808
as.numeric(get(snpname))	-0.07749967	0.1806078	-0.4291047	0.6678860

Testing association between qt and SNP snp3 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.06382773	0.05629376	1.1338331	0.2569789
as.numeric(get(snpname))	-0.02517149	0.02894125	-0.8697443	0.3845280

Testing association between qt and SNP snp4 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.14005988	0.05612775	2.495377	0.01264938
as.numeric(get(snpname))	-0.07284539	0.02982557	-2.442380	0.01466282

Testing association between qt and SNP snp5 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.14350846	0.06620992	-2.167477	0.03029734
as.numeric(get(snpname))	0.07404874	0.02941437	2.517434	0.01188645

Testing association between qt and SNP snp6 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.12737489	0.06768096	-1.881990	0.05995937
as.numeric(get(snpname))	0.06724115	0.02924840	2.298969	0.02159304

Testing association between qt and SNP snp7 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.08884244	0.05475991	1.622399	0.1048511
as.numeric(get(snpname))	-0.03774136	0.03152701	-1.197112	0.2313829

Testing association between qt and SNP snp8 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.05214665	0.08116533	-0.6424744	0.5206274
as.numeric(get(snpname))	0.06942327	0.07222881	0.9611576	0.3365710

Testing association between qt and SNP snp9 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.2201742	0.07115711	-3.094199	0.0019965937
as.numeric(get(snpname))	0.2110978	0.06112112	3.453761	0.0005625794

Testing association between qt and SNP snp10 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.01474695	0.05749473	-0.2564921	0.7975930
as.numeric(get(snpname))	0.03140888	0.04251458	0.7387789	0.4601141

**Answer (Ex. 14)** — Generally, results do not change much: still, SNPs 1, 4, 5, 6 and 9 are significantly associated, and  $p$ -values are close to these observed without adjustment For SNPs

```
> for (i in 1:10) {
```

```
+ snpname <- paste("snp",i,sep="")
+ cat("\nTesting sex-adjusted association between qt and SNP",snpname,":\n")
+ testmodel <- lm(qt~sex+as.numeric(get(snpname)))
+ print(summary(testmodel)$coef)
+ }
```

Testing sex-adjusted association between qt and SNP snp1 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.12783200	0.05660623	-2.2582672	0.024019551
sex	0.01766209	0.04061074	0.4349117	0.663666095
as.numeric(get(snpname))	0.08868826	0.03148302	2.8170191	0.004887301

Testing sex-adjusted association between qt and SNP snp2 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.08169692	0.18525892	0.4409878	0.6592621
sex	0.01977118	0.04101612	0.4820343	0.6298261
as.numeric(get(snpname))	-0.07770464	0.18063757	-0.4301688	0.6671120

Testing sex-adjusted association between qt and SNP snp3 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.05518829	0.06028776	0.9154146	0.3600669
sex	0.01626276	0.04057000	0.4008568	0.6885616
as.numeric(get(snpname))	-0.02491788	0.02895328	-0.8606239	0.3895321

Testing sex-adjusted association between qt and SNP snp4 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.12611774	0.06040009	2.0880388	0.03690009
sex	0.02553529	0.04083273	0.6253633	0.53179244
as.numeric(get(snpname))	-0.07227905	0.02984312	-2.4219671	0.01551079

Testing sex-adjusted association between qt and SNP snp5 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.15272175	0.06880690	-2.2195704	0.02654187
sex	0.02009534	0.04076087	0.4930057	0.62205408
as.numeric(get(snpname))	0.07357452	0.02943476	2.4995791	0.01250094

Testing sex-adjusted association between qt and SNP snp6 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.14110680	0.07002874	-2.0149841	0.04401871
sex	0.03117861	0.04077551	0.7646406	0.44456146
as.numeric(get(snpname))	0.06634602	0.02927437	2.2663517	0.02351939

Testing sex-adjusted association between qt and SNP snp7 :

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.06697438	0.05879604	1.139097	0.2547782
sex	0.04174468	0.04087001	1.021401	0.3071689
as.numeric(get(snpname))	-0.03723286	0.03153066	-1.180846	0.2377825

Testing sex-adjusted association between qt and SNP snp8 :

	Estimate	Std. Error	t value	Pr(> t )
--	----------	------------	---------	----------

```
(Intercept)          -0.06807682 0.08455606 -0.8051087 0.4208378
sex                   0.02760968 0.04102873  0.6729354 0.5010541
as.numeric(get(snpname)) 0.07124407 0.07228780  0.9855614 0.3244491
```

Testing sex-adjusted association between qt and SNP snp9 :

```
              Estimate Std. Error    t value    Pr(>|t|)
(Intercept)   -0.22558672 0.07313030  -3.0847229 0.0020610506
sex            0.01311497 0.04074702   0.3218632 0.7475848790
as.numeric(get(snpname)) 0.21002414 0.06122367   3.4304402 0.0006129721
```

Testing sex-adjusted association between qt and SNP snp10 :

```
              Estimate Std. Error    t value    Pr(>|t|)
(Intercept)   -0.02805887 0.06107457  -0.4594198 0.6459747
sex            0.02628396 0.04064009   0.6467496 0.5178563
as.numeric(get(snpname)) 0.03150834 0.04252005   0.7410231 0.4587525
```

**Answer (Ex. 15)** — SNPs 1, 4, 5 and 9 are significantly associated at nominal  $P \leq 0.05$ . SNP 6 is only marginally significantly associated under the general genotypic model. Here is a testing script (no need to reproduce that, just check the results):

```
> for (i in 1:10) {
+   snpname <- paste("snp", i, sep="")
+   cat("\nTesting association between qt and SNP", snpname, "\n")
+   print(anova(lm(qt~get(snpname)), test="Chisq"))
+   #print(summary(lm(qt~get(snpname)))$coef)
+ }
```

Testing association between qt and SNP snp1 :  
Analysis of Variance Table

Response: qt

```
              Df Sum Sq Mean Sq F value    Pr(>F)
get(snpname)    2    7.8   3.8995   3.9845 0.01873 *
Residuals  2371 2320.4   0.9787
---
```

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Testing association between qt and SNP snp2 :  
Analysis of Variance Table

Response: qt

```
              Df Sum Sq Mean Sq F value    Pr(>F)
get(snpname)    1    0.18 0.18376   0.1841 0.6679
Residuals  2372 2367.23 0.99799
```

Testing association between qt and SNP snp3 :  
Analysis of Variance Table

Response: qt

```

              Df  Sum Sq Mean Sq F value Pr(>F)
get(snpname)    2    3.24  1.61986   1.658 0.1907
Residuals      2375 2320.41  0.97701

```

Testing association between qt and SNP snp4 :  
Analysis of Variance Table

Response: qt

```

              Df  Sum Sq Mean Sq F value  Pr(>F)
get(snpname)    2    7.68  3.8417  3.8628 0.02114 *
Residuals      2387 2373.94  0.9945

```

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Testing association between qt and SNP snp5 :  
Analysis of Variance Table

Response: qt

```

              Df  Sum Sq Mean Sq F value  Pr(>F)
get(snpname)    2    6.48  3.2418  3.2798 0.03781 *
Residuals      2380 2352.48  0.9884

```

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Testing association between qt and SNP snp6 :  
Analysis of Variance Table

Response: qt

```

              Df  Sum Sq Mean Sq F value  Pr(>F)
get(snpname)    2    5.49  2.74680  2.7808 0.06219 .
Residuals      2377 2347.91  0.98776

```

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Testing association between qt and SNP snp7 :  
Analysis of Variance Table

Response: qt

```

              Df  Sum Sq Mean Sq F value  Pr(>F)
get(snpname)    2    4.02  2.01212  2.0368 0.1307
Residuals      2365 2336.31  0.98787

```

Testing association between qt and SNP snp8 :  
Analysis of Variance Table

Response: qt

```

              Df  Sum Sq Mean Sq F value  Pr(>F)
get(snpname)    2    1.38  0.68987  0.6924 0.5005
Residuals      2368 2359.23  0.99630

```

Testing association between qt and SNP snp9 :  
Analysis of Variance Table

Response: qt

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
get(snpname)	2	15.6	7.8014	7.9982	0.0003453 ***
Residuals	2358	2300.0	0.9754		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Testing association between qt and SNP snp10 :  
Analysis of Variance Table

Response: qt

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
get(snpname)	2	1.19	0.59456	0.6041	0.5467
Residuals	2381	2343.47	0.98424		

**Answer (Ex. 16)** — For 'snp1', though the data are compatible with either additive, dominant or recessive model, the additive model provides best fit to the data (largest  $p$ -value), while the recessive 'B' model provide the worst fit (almost significantly worse than the general model):

```
> table(snp1, as.numeric(snp1))
snp1      1      2      3
  A/A 1287      0      0
  A/B   0  888      0
  B/B   0   0  199

> table(snp1, (as.numeric(snp1)>=2))
snp1 FALSE TRUE
  A/A 1287    0
  A/B   0  888
  B/B   0  199

> table(snp1, (as.numeric(snp1)>=3))
snp1 FALSE TRUE
  A/A 1287    0
  A/B  888    0
  B/B   0  199

> model_gen <- lm(qt~snp1)
> summary(model_gen)
```

Call:

```
lm(formula = qt ~ snp1)
```

Residuals:

Min	1Q	Median	3Q	Max
-3.5261	-0.6643	-0.0111	0.6765	3.5462

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.02846	0.02758	-1.032	0.3022
snp1A/B	0.08200	0.04316	1.900	0.0575 .
snp1B/B	0.18644	0.07536	2.474	0.0134 *

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.9893 on 2371 degrees of freedom

(126 observations deleted due to missingness)

Multiple R-squared: 0.00335, Adjusted R-squared: 0.002509

F-statistic: 3.985 on 2 and 2371 DF, p-value: 0.01873

```
> model_add <- lm(qt~as.numeric(snp1))
> model_dom <- lm(qt~I(as.numeric(snp1)>=2))
> model_rec <- lm(qt~I(as.numeric(snp1)>=3))
> anova(model_add,model_gen,test="Chisq")
```

Analysis of Variance Table

Model 1: qt ~ as.numeric(snp1)

Model 2: qt ~ snp1

	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
1	2372	2320.5			
2	2371	2320.4	1	0.04886	0.8232

```
> anova(model_dom,model_gen,test="Chisq")
```

Analysis of Variance Table

Model 1: qt ~ I(as.numeric(snp1) >= 2)

Model 2: qt ~ snp1

	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
1	2372	2322.2			
2	2371	2320.4	1	1.7733	0.1783

```
> anova(model_rec,model_gen,test="Chisq")
```

Analysis of Variance Table

Model 1: qt ~ I(as.numeric(snp1) >= 3)

Model 2: qt ~ snp1

	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
1	2372	2324.0			
2	2371	2320.4	1	3.5332	0.05743 .

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

For SNPs 4, 5, 6, and 9 results are:

```
> for (i in c(4,5,6,9)) {
+   snpname <- paste("snp",i,sep="")
+   cat("\nTesting SNP",snpname,":\n")
+   cursnp <- get(snpname)
+   model_gen <- lm(qt~cursnp)
```

```
+      print(summary(model_gen))
+      model_add <- lm(qt~as.numeric(cursnp))
+      model_dom <- lm(qt~I(as.numeric(cursnp)>=2))
+      model_rec <- lm(qt~I(as.numeric(cursnp)>=3))
+      print(anova(model_add,model_gen,test="Chisq"))
+      print(anova(model_dom,model_gen,test="Chisq"))
+      print(anova(model_rec,model_gen,test="Chisq"))
+    }
```

Testing SNP snp4 :

Call:

```
lm(formula = qt ~ cursnp)
```

Residuals:

	Min	1Q	Median	3Q	Max
	-3.4311	-0.6636	-0.0013	0.6737	3.5489

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.02132	0.02972	0.717	0.4733
cursnpA/A	0.02953	0.04423	0.668	0.5044
cursnpB/B	-0.14481	0.06192	-2.339	0.0194 *

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.9973 on 2387 degrees of freedom

(110 observations deleted due to missingness)

Multiple R-squared: 0.003226, Adjusted R-squared: 0.002391

F-statistic: 3.863 on 2 and 2387 DF, p-value: 0.02114

Analysis of Variance Table

Model 1: qt ~ as.numeric(cursnp)

Model 2: qt ~ cursnp

	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
1	2388	2375.7			
2	2387	2373.9	1	1.7489	0.1848

Analysis of Variance Table

Model 1: qt ~ I(as.numeric(cursnp) >= 2)

Model 2: qt ~ cursnp

	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
1	2388	2379.4			
2	2387	2373.9	1	5.4391	0.01936 *

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Table

Model 1: qt ~ I(as.numeric(cursnp) >= 3)



Model 2: qt ~ cursnp

	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
1	2388	2374.4			
2	2387	2373.9	1	0.44342	0.5043

Testing SNP snp5 :

Call:

lm(formula = qt ~ cursnp)

Residuals:

	Min	1Q	Median	3Q	Max
	-3.4719	-0.6589	-0.0084	0.6622	3.5285

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.01401	0.02878	0.487	0.6264
cursnpA/A	-0.09667	0.05611	-1.723	0.0851 .
cursnpB/B	0.05727	0.04607	1.243	0.2140

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.9942 on 2380 degrees of freedom

(117 observations deleted due to missingness)

Multiple R-squared: 0.002749, Adjusted R-squared: 0.00191

F-statistic: 3.28 on 2 and 2380 DF, p-value: 0.03781

Analysis of Variance Table

Model 1: qt ~ as.numeric(cursnp)

Model 2: qt ~ cursnp

	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
1	2381	2352.7			
2	2380	2352.5	1	0.22152	0.6359

Analysis of Variance Table

Model 1: qt ~ I(as.numeric(cursnp) >= 2)

Model 2: qt ~ cursnp

	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
1	2381	2354.0			
2	2380	2352.5	1	1.5273	0.2138

Analysis of Variance Table

Model 1: qt ~ I(as.numeric(cursnp) >= 3)

Model 2: qt ~ cursnp

	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
1	2381	2355.4			
2	2380	2352.5	1	2.9335	0.08494 .

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Testing SNP snp6 :

Call:

```
lm(formula = qt ~ cursnp)
```

Residuals:

Min	1Q	Median	3Q	Max
-3.4784	-0.6753	-0.0064	0.6703	3.5324

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.07617	0.05085	-1.498	0.1343
cursnpB/A	0.09417	0.05886	1.600	0.1097
cursnpB/B	0.14351	0.06096	2.354	0.0186 *

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.9939 on 2377 degrees of freedom

(120 observations deleted due to missingness)

Multiple R-squared: 0.002334, Adjusted R-squared: 0.001495

F-statistic: 2.781 on 2 and 2377 DF, p-value: 0.06219

Analysis of Variance Table

Model 1: qt ~ as.numeric(cursnp)

Model 2: qt ~ cursnp

	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
1	2378	2348.2			
2	2377	2347.9	1	0.27462	0.598

Analysis of Variance Table

Model 1: qt ~ I(as.numeric(cursnp) >= 2)

Model 2: qt ~ cursnp

	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
1	2378	2349.1			
2	2377	2347.9	1	1.1967	0.271

Analysis of Variance Table

Model 1: qt ~ I(as.numeric(cursnp) >= 3)

Model 2: qt ~ cursnp

	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
1	2378	2350.4			
2	2377	2347.9	1	2.5284	0.1096

Testing SNP snp9 :

Call:

```
lm(formula = qt ~ cursnp)
```

Residuals:

Min	1Q	Median	3Q	Max
-3.5482	-0.6673	0.0074	0.6546	3.6061

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.006298	0.021562	-0.292	0.77026
cursnpA/B	0.162230	0.065729	2.468	0.01365 *
cursnpB/B	1.002439	0.313057	3.202	0.00138 **

---  
Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.9876 on 2358 degrees of freedom

(139 observations deleted due to missingness)

Multiple R-squared: 0.006738, Adjusted R-squared: 0.005896

F-statistic: 7.998 on 2 and 2358 DF, p-value: 0.0003453

Analysis of Variance Table

Model 1: qt ~ as.numeric(cursnp)

Model 2: qt ~ cursnp

	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
1	2359	2303.9			
2	2358	2300.0	1	3.9528	0.04411 *

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Table

Model 1: qt ~ I(as.numeric(cursnp) >= 2)

Model 2: qt ~ cursnp

	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
1	2359	2306.8			
2	2358	2300.0	1	6.7911	0.008324 **

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Table

Model 1: qt ~ I(as.numeric(cursnp) >= 3)

Model 2: qt ~ cursnp

	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
1	2359	2305.9			
2	2358	2300.0	1	5.942	0.01358 *

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1



## Chapter 4

# Introduction to GenABEL-package

In this section, you will become familiar with the **GenABEL-package** library, designed for GWA analysis. Compared to **genetics** package, it provides specific facilities for storage and manipulation of large amounts of data, very fast tests for GWA analysis, and special functions to analyse and graphically present the results of GWA analysis (thus "analysis of analysis").

Start R and load **GenABEL-package** library using command

```
> library(GenABEL)
```

After that, load example data set using the command

```
> data(srdta)
```

### 4.1 General description of `gwaa.data-class`

The object you have loaded, `srdta`, belongs to the `gwaa.data` class. This is a special class developed to facilitate GWA analysis.

In GWA analysis, different types of data are used. These include the phenotypic and genotypic data on the study participants and chromosome and location of every SNP. For every SNP, it is desirable to know the details of coding (what are alleles? – A, T, G, C? – and what is the strand – '+' or '-', 'top' or 'bot'? – this coding is for).

One could attempt to store all phenotypes and genotypes together in a single table, using, e.g. one row per study subject; than the columns will correspond to study phenotypes and SNPs. For a typical GWA data set, this would lead to a table of few thousands rows and few hundreds of thousands to millions of columns. Such a format is generated when one downloads HapMap data for a region. To store GWA data in such tables internally, within R, proves to be inefficient. In **GenABEL-package**, special data class, `gwaa.data-class` is used to store GWA data.

You may consider an object of `gwaa.data-class` as a 'black box' from which you can get specific data using specific functions. If you are interested in internal

structure of the `gwaa.data-class`, you can find the description in section B.1 (Internal structure of `gwaa.data-class`).

The data frame, which contains all phenotypic data in the study may be accessed using the `phdata` function. Let us have a look at few first rows of the phenotypic data frame of `srdta`:

```
> phdata(srdta)[1:5,]
      id sex  age  qt1  qt2 qt3 bt
p1 p1   1 43.4 -0.58  4.46 1.43  0
p2 p2   1 48.2  0.80  6.32 3.90  1
p3 p3   0 37.9 -0.52  3.26 5.05  1
p4 p4   1 53.8 -1.55 888.00 3.76  1
p5 p5   1 47.5  0.25  5.70 2.89  1
```

The rows of this data frame correspond to study subjects, and the columns correspond to the variables. There are two default variables, which are always present in `phdata`. The first of these is "id", which contains study subject identification code. This identification code can be arbitrary character, numer, or alphanumeric combination, but every person must be coded with a unique ID. The second default variable is "sex", where males are coded with ones ("1") and females are coded with zero ("0").

It is important to understand that this data frame is not supposed to be directly modified by the user, as its structure is coupled to the structure of genotypic data. If at some point you need to manipulate (add/delete) the phenotypes included in `phdata`, you need to use such `GenABEL-package` functions as `add.phdata` and `del.phdata` (see section 4.2).

The other part of an object of `gwaa.data-class` is `gtdata`, which contains all GWA genetic information in an object of class `snps.data` class. It is not supposed to be modified directly by user. The genotypic data can be accessed through `gtdata` function, e.g.

```
> gtdata(srdta[1:10,1:10])

@nids = 10
@nsnps = 10
@nbytes = 3
@idnames = p1 p2 p3 p4 p5 p6 p7 p8 p9 p10
@snpsnames = rs10 rs18 rs29 rs65 rs73 rs114 rs128 rs130 rs143 rs150
@chromosome = 1 1 1 1 1 1 1 1 1 1
@coding = 08 0b 0c 03 04 03 0c 04 08 0f
@strand = 01 01 02 01 01 01 02 01 01 01
@map = 2500 3500 5750 13500 14250 24500 27000 27250 31000 33250
@male = 1 1 0 1 1 0 0 1 0 0
@gtps =
40 40 40 80 40 40 40 40 c0 c0
40 40 00 00 40 40 40 c0 40 40
40 40 00 80 40 40 40 40 c0 c0
```

As you can see, these data are of little direct use as these are stored in an internal format – you need to coerce that to other data type if you want to manipulate / analyse these data using non-`GenABEL-package` functions (see section ??).

The number of individuals described in an object of `gwaa.data-class` can be accessed through `nids` function, e.g.

```
> nids(srdta)

[1] 2500
```

and the number of SNPs using the `nsnps` function:

```
> nsnps(srdta)

[1] 833
```

The IDs of individuals included in the study can be accessed via `idnames` function, for example IDs of the first 7 individuals in the study are

```
> idnames(srdta)[1:7]

[1] "p1" "p2" "p3" "p4" "p5" "p6" "p7"
```

The sex of the individuals can be accessed using the `male` function:

```
> male(srdta)[1:7]

p1 p2 p3 p4 p5 p6 p7
 1  1  0  1  1  0  0
```

where males (heterogametic sex) are assigned with '1' and a homogametic sex (females) are assigned value '0'.

Names of SNPs can be accessed using `snpnames` function; for example the names of the first 10 SNPs in the `srdta` are

```
> snpnames(srdta)[1:10]

[1] "rs10" "rs18" "rs29" "rs65" "rs73" "rs114" "rs128" "rs130" "rs143"
[10] "rs150"
```

SNP annotation include (presented for the first 10 SNPs only):

- Chromosome:

```
> chromosome(srdta)[1:10]

rs10 rs18 rs29 rs65 rs73 rs114 rs128 rs130 rs143 rs150
"1"  "1"  "1"  "1"  "1"  "1"  "1"  "1"  "1"  "1"
```

- Map position

```
> map(srdta)[1:10]

rs10 rs18 rs29 rs65 rs73 rs114 rs128 rs130 rs143 rs150
2500 3500 5750 13500 14250 24500 27000 27250 31000 33250
```

- Coding (where the second allele is the "effect" or "coded" one):

```
> coding(srdta)[1:10]
```

```
rs10 rs18 rs29 rs65 rs73 rs114 rs128 rs130 rs143 rs150
"TG" "GA" "GT" "AT" "AG" "AT" "GT" "AG" "TG" "CA"
```

For every SNP, coding is presented with a pair of characters, for example "AG". For 'AG' polymorphism, you may expect "AA", "AG" and "GG" genotypes to be found in population. The order (that is "AG" vs "GA") is important – the first allele reported is the one which will be used as a reference in association analysis, and thus the effects are reported for the second allele. You can also access the reference allele with the method `refallele`

```
> refallele(srdta)[1:10]
```

```
rs10 rs18 rs29 rs65 rs73 rs114 rs128 rs130 rs143 rs150
"T" "G" "G" "A" "A" "A" "G" "A" "T" "C"
```

and the effective (or 'coded') allelel with

```
> effallele(srdta)[1:10]
```

```
rs10 rs18 rs29 rs65 rs73 rs114 rs128 rs130 rs143 rs150
"G" "A" "T" "T" "G" "T" "T" "G" "G" "A"
```

- Strand on which the coding is reported ('+', '-' or missing, 'u'):

```
> strand(srdta)[1:10]
```

```
rs10 rs18 rs29 rs65 rs73 rs114 rs128 rs130 rs143 rs150
"+" "+" "-" "+" "+" "+" "-" "+" "+" "+"
```

#### Summary:

- GenABEL-package uses special data class, `gwaa.data-class`, to store GWA data.
- To access the content of an object of `gwaa.data-class`, a number of functions is used

#### Exercise 1. Exploring IDs in `srdta`

Explore `srdta`.

1. How many people are included in the study?
2. How many of these are males?
3. How many are females?
4. What is male proportion?



**Exercise 2. Exploring SNPs in `srdta`**

Explore SNPs contained in `srdta` using functions to access SNP names (`snpnames`) and map (`map`) location

1. What are names of markers located after 2,490,000 b.p.?
2. Between 1,100,000 and 1,105,000 b.p.?

**4.2 Accessing and modifying phenotypic data**

As it was already mentioned, the object returned by `phdata` contains phenotypic data and is an conventional data frame, wich obligatory includes 'id' and 'sex' variables, and ordered an a way that it couples to the genotypic data.

Being a data frame, `phdata` may be accessed using corresponding methods:

```
> phdata(srdta)[1:5,]

  id sex  age  qt1  qt2 qt3 bt
p1 p1   1 43.4 -0.58  4.46 1.43  0
p2 p2   1 48.2  0.80  6.32 3.90  1
p3 p3   0 37.9 -0.52  3.26 5.05  1
p4 p4   1 53.8 -1.55 888.00 3.76  1
p5 p5   1 47.5  0.25  5.70 2.89  1

> class(phdata(srdta))

[1] "data.frame"

> phdata(srdta)[1:5,2]

[1] 1 1 0 1 1

> phdata(srdta)[1:5,"sex"]

[1] 1 1 0 1 1

> phdata(srdta)$sex[1:5]

[1] 1 1 0 1 1
```

The modification of the phenotypic data is performed using special methods, because of specific restrictions on phenotypic data frames. There are two main functions which allow you to add (`add.phdata`) and delete (`del.phdata`) phenotypes from `phdata` part of an object of `gwaa.data-class`.

For example, if you want to add a variable (say, square of age) computed from the 'age' variable of `srdta`

```
> phdata(srdta)[1:5,]

  id sex  age  qt1  qt2 qt3 bt
p1 p1   1 43.4 -0.58  4.46 1.43  0
p2 p2   1 48.2  0.80  6.32 3.90  1
p3 p3   0 37.9 -0.52  3.26 5.05  1
p4 p4   1 53.8 -1.55 888.00 3.76  1
p5 p5   1 47.5  0.25  5.70 2.89  1
```

```
> age2 <- phdata(srdta)$age^2
```

you need to use `add.phdata` function:

```
> srdta <- add.phdata(srdta,newph=age2,name="age_squared")
> phdata(srdta)[1:5,]
```

	id	sex	age	qt1	qt2	qt3	bt	age_squared
p1	p1	1	43.4	-0.58	4.46	1.43	0	1883.56
p2	p2	1	48.2	0.80	6.32	3.90	1	2323.24
p3	p3	0	37.9	-0.52	3.26	5.05	1	1436.41
p4	p4	1	53.8	-1.55	888.00	3.76	1	2894.44
p5	p5	1	47.5	0.25	5.70	2.89	1	2256.25

You can add more then one variable at once using the same function, however, in this case the second ('newph') argument of the function should be a data frame, which contains 'id' variable specifying the IDs of individuals. Imagine we have the data for individuals 'p1', 'p2' and 'p7' (we will generate random data for them; pay attention only to the result):

```
> newvalues <- matrix(rnorm(3*5),3,5)
> newdata <- data.frame(id=c("p1","p2","p7"),ph1=1,ph2=1,ph3=1,ph4=1,ph5=1)
> newdata[,c(2:6)] <- newvalues
> newdata
```

	id	ph1	ph2	ph3	ph4	ph5
1	p1	0.5985878	-1.136133	-1.682711453	-0.7420314	-0.00644069
2	p2	-1.5182653	1.974455	0.552566806	-0.2659583	0.41389476
3	p7	-0.1186581	1.713226	-0.006493404	-0.3150254	1.54981357

These data can be added to phenotypic data with

```
> srdta <- add.phdata(srdta,newdata)
> phdata(srdta)[1:10,]
```

	id	sex	age	qt1	qt2	qt3	bt	age_squared	ph1	ph2
p1	p1	1	43.4	-0.58	4.46	1.43	0	1883.56	0.5985878	-1.136133
p2	p2	1	48.2	0.80	6.32	3.90	1	2323.24	-1.5182653	1.974455
p3	p3	0	37.9	-0.52	3.26	5.05	1	1436.41	NA	NA
p4	p4	1	53.8	-1.55	888.00	3.76	1	2894.44	NA	NA
p5	p5	1	47.5	0.25	5.70	2.89	1	2256.25	NA	NA
p6	p6	0	45.0	0.15	4.65	1.87	0	2025.00	NA	NA
p7	p7	0	52.0	-0.56	4.64	2.49	0	2704.00	-0.1186581	1.713226
p8	p8	1	42.5	NA	5.77	2.68	1	1806.25	NA	NA
p9	p9	0	29.7	-2.26	0.71	1.45	0	882.09	NA	NA
p10	p10	0	45.8	-1.32	3.26	0.85	0	2097.64	NA	NA

	ph3	ph4	ph5
p1	-1.682711453	-0.7420314	-0.00644069
p2	0.552566806	-0.2659583	0.41389476
p3	NA	NA	NA
p4	NA	NA	NA
p5	NA	NA	NA

```

p6      NA      NA      NA
p7 -0.006493404 -0.3150254 1.54981357
p8      NA      NA      NA
p9      NA      NA      NA
p10     NA      NA      NA

```

Finally, if you need, you can delete some phenotypes from the `phdata` using `del.phdata` function. Let us delete the phenotypes we have just added:

```

> srdta <- del.phdata(srdta, c("age_squared", "ph1", "ph2", "ph3", "ph4", "ph5"))
> phdata(srdta)[1:10,]

```

```

      id sex age qt1 qt2 qt3 bt
p1  p1  1 43.4 -0.58 4.46 1.43 0
p2  p2  1 48.2 0.80 6.32 3.90 1
p3  p3  0 37.9 -0.52 3.26 5.05 1
p4  p4  1 53.8 -1.55 888.00 3.76 1
p5  p5  1 47.5 0.25 5.70 2.89 1
p6  p6  0 45.0 0.15 4.65 1.87 0
p7  p7  0 52.0 -0.56 4.64 2.49 0
p8  p8  1 42.5 NA 5.77 2.68 1
p9  p9  0 29.7 -2.26 0.71 1.45 0
p10 p10 0 45.8 -1.32 3.26 0.85 0

```

#### Summary:

- Phenotypic data contained in an object of `gwaa.data-class` can be accessed using `phdata` functions
- You can add phenotypes using `add.phdata` function
- You can delete phenotypes using `del.phdata` function

## 4.3 Sub-setting and coercing gwaa.data

It is possible to sub-set the object, which stores the GWA data in the manner similar to that used for conventional R matrices and data frames. Very primitively, you may think of an object of class `gwaa.data` as a matrix whose rows correspond to study subjects and columns correspond to SNPs studied (though the actual object is a way more complicated). For example, if we would like to investigate what is the content of `srdta` for the first 5 people and 3 SNPs, we can run

```

> ssubs <- srdta[1:5,1:3]
> class(ssubs)

[1] "gwaa.data"
attr(,"package")
[1] "GenABEL"

```

As you can see, by sub-setting we obtained a smaller object of `gwaa.data-class`. The two major parts it contains are phenotypic data, which can be accessed through `phdata` (discussed in section 4.2):

```
> phdata(ssubs)

      id sex  age  qt1    qt2 qt3 bt
p1 p1   1 43.4 -0.58  4.46 1.43  0
p2 p2   1 48.2  0.80  6.32 3.90  1
p3 p3   0 37.9 -0.52  3.26 5.05  1
p4 p4   1 53.8 -1.55 888.00 3.76  1
p5 p5   1 47.5  0.25  5.70 2.89  1
```

and genotypic data, which can be accessed via `gtdata` function:

```
> gtdata(ssubs)

@nids = 5
@nsnps = 3
@nbytes = 2
@idnames = p1 p2 p3 p4 p5
@snpsnames = rs10 rs18 rs29
@chromosome = 1 1 1
@coding = 08 0b 0c
@strand = 01 01 02
@map = 2500 3500 5750
@male = 1 1 0 1 1
@gtps =
40 40 40
40 40 00
```

whose content is not quite straightforward to read.

To get human-readable information, genotypic object should be coerced to a regular R data type, e.g. character, using `as.character()` function:

```
> as.character(gtdata(ssubs))

      rs10 rs18 rs29
p1 "T/T" "G/G" "G/G"
p2 "T/T" "G/G" NA
p3 "T/T" "G/G" NA
p4 "T/T" "G/G" NA
p5 "T/T" "G/A" "G/G"
```

Other useful coercion is to "numeric":

```
> as.numeric(gtdata(ssubs))

      rs10 rs18 rs29
p1      0      0      0
p2      0      0     NA
p3      0      0     NA
p4      0      0     NA
p5      0      1      0
```

Note that conversion to numeric happened according to the underlying genotype and the rules specified by SNP coding:

```
> coding(ssubs)

rs10 rs18 rs29
"TG" "GA" "GT"
```

– the genotype, which is made of the 'first' allele of the 'code' is converted to 0, the heterozygote to '1' and a homozygote for the second allele is converted to '2'.

For example, when coding is "GA", as is for the `rs18` (the second SNP), homozygotes for the first allele, as specified by coding ("G") are converted to zeros ("0"), heterozygotes are converted to ones ("1"), and homozygotes for the second allele ("A") are converted to twos ("2"). Clearly, when numerically converted data are used for association analysis, the effects will be estimated for the second allele, while first will be used as a reference.

Genotypic data converted to standard R format can be used in any further analysis.

Several useful genetic analysis libraries were developed for R. These include `genetics` (analysis of linkage disequilibrium and many other useful functions) and `haplo.stats` (analysis of association between traits and haplotypes). These use their own genetic data formats.

One can translate `GenABEL`-package genetic data to the format used by "genetics" library by `as.genotype()`:

```
> as.genotype(gtdata(ssubs))
```

NOTE: THIS PACKAGE IS NOW OBSOLETE.

The R-Genetics project has developed an set of enhanced `genetics` packages to replace 'genetics'. Please visit the project homepage at <http://rgenetics.org> for information.

```
rs10 rs18 rs29
p1 T/T G/G G/G
p2 T/T G/G <NA>
p3 T/T G/G <NA>
p4 T/T G/G <NA>
p5 T/T G/A G/G
```

To translate `GenABEL`-package data to the format used by "haplo.stats" you can use function `as.hsgeno()`

```
> as.hsgeno(gtdata(ssubs))

rs10.a1 rs10.a2 rs18.a1 rs18.a2 rs29.a1 rs29.a2
p1      1      1      1      1      1      1
p2      1      1      1      1     NA     NA
p3      1      1      1      1     NA     NA
p4      1      1      1      1     NA     NA
p5      1      1      1      2      1      1
```

Actually, most users will not need the latter function, as **GenABEL-package** provides a functional interface to "haplo.stats" (such **GenABEL-package** functions as `scan.haplo()` and `scan.haplo.2D()`).

It is possible to select sub-sets of **gwaa.data-class** based not only on index (e.g. first 10 people and SNP number 33), but also based on names.

For example, if we would like to retrieve phenotypic data on people with IDs "p141", "p147" and "p2000", we can use

```
> phdata(srdta[c("p141", "p147", "p2000"),])
```

	id	sex	age	qt1	qt2	qt3	bt
p141	p141	0	47.2	0.51	5.23	2.17	0
p147	p147	0	43.2	0.14	4.47	1.73	0
p2000	p2000	0	43.1	-1.53	2.78	2.70	1

here, the first part of expression sub-sets **srdta** on selected IDs, and the second tells which part of the retrieved sub-set we want to see. You can try `srdta[c("p141", "p147", "p2000"),]`, but be prepared to see long output, as all information will be reported.

In similar manner, we can also select on SNP name. For example, if we are interested to see information on SNPs "rs10" and "rs29" for above people, we can run

```
> phdata(srdta[c("p141", "p147", "p2000"), c("rs10", "rs29")])
```

	id	sex	age	qt1	qt2	qt3	bt
p141	p141	0	47.2	0.51	5.23	2.17	0
p147	p147	0	43.2	0.14	4.47	1.73	0
p2000	p2000	0	43.1	-1.53	2.78	2.70	1

```
> gtdata(srdta[c("p141", "p147", "p2000"), c("rs10", "rs29")])
```

```
@nids = 3
@nsnps = 2
@nbytes = 1
@idnames = p141 p147 p2000
@snpsnames = rs10 rs29
@chromosome = 1 1
@coding = 08 0c
@strand = 01 02
@map = 2500 5750
@male = 0 0 0
@gtps =
40 40
```

To see the actual genotypes for the above three people and two SNPs, use

```
> as.character(srdta[c("p141", "p147", "p2000"), c("rs10", "rs29")])
```

	rs10	rs29
p141	"T/T"	"G/G"
p147	"T/T"	"G/G"
p2000	"T/G"	"G/T"

or

```
> as.numeric(srdta[c("p141", "p147", "p2000"), c("rs10", "rs29")])
```

	rs10	rs29
p141	0	0
p147	0	0
p2000	1	1

### Exercise 3. Exploring rs114

Explore genotypes for SNP "rs114"

1. What is the coding and which allele is the reference one?
2. What is the frequency of **non-reference** ("effective") allele in total sample?
3. What is the frequency of effective allele in male?
4. What is the frequency of effective allele in female?
5. What is the frequency of the **reference** allele in total sample, males and females?

#### Summary:

- It is possible to obtain subsets of objects of `gwaa.data-class` and `snp.data-class` using standard 2D sub-setting model `[i,j]`, where `i` corresponds to study subjects and `j` corresponds to SNPs.
- It is possible to provide ID and SNP names instead of indexes when sub-setting an object of class `gwaa.data-class`.
- Function `as.numeric()` converts genotypic data from `snp.data-class` to regular integer numbers, which can be used in analysis with R.
- Function `as.character()` converts genotypic data from `snp.data-class` to character format.
- Function `as.genotype()` converts genotypic data from `snp.data-class` to the format used by library `genetics`.
- Function `as.hsgeno()` converts genotypic data from `snp.data-class` to the format used by library `haplo.stats`.

## 4.4 Exploring genetic data

Implementation of function `summary()` to summarize genotypic part of `gwaa.data-class` is very useful in genetic data exploration and quality control (QC). Let us try application of this function to the `ssubs`:

```
> a <- summary(ssubs)
> a
```

	Chromosome	Position	Strand	A1	A2	NoMeasured	CallRate	Q.2	P.11	P.12	P.22
rs10	1	2500	+	T	G	5	1.0	0.0	5	0	0
rs18	1	3500	+	G	A	5	1.0	0.1	4	1	0
rs29	1	5750	-	G	T	2	0.4	0.0	2	0	0

	Pexact	Fmax	Plrt
rs10	1	0.0000000	1.0000000
rs18	1	-0.1111111	0.7386227
rs29	1	0.0000000	1.0000000

In the first section, the summary is generated for phenotypic data. In the second section, summary is generated for genotypic data. In this section, **NoMeasured** refers to the number of genotypes scores, **CallRate** to the proportion of these, **Q.2** is the frequency of the 'B' allele. The counts in three genotypic classes are provided next. **Pexact** refers to exact P-value for the test of Hardy-Weinberg equilibrium.

As you've seen above, an object of the class **gwaa.data-class** is sub-settable in standard manner: **[i,j]**, where **i** is an index of a study subject and **j** is an index of a SNP. Importantly, **i** could be a list of indexes:

```
> vec <- which(phdata(srdta)$age>=65)
> vec
```

```
[1] 64 122 186 206 207 286 385 386 492 514 525 536 545 565 613
[16] 632 649 673 701 779 799 981 1008 1131 1186 1223 1281 1383 1471 1489
[31] 1501 1565 1584 1673 1679 1782 1821 1832 1866 1891 1953 2081 2085 2140 2224
[46] 2268 2291 2384 2420 2453
```

```
> summary(gtdata(srdta[vec,1:3]))
```

	Chromosome	Position	Strand	A1	A2	NoMeasured	CallRate	Q.2	P.11	P.12
rs10	1	2500	+	T	G	48	0.96	0.1354167	36	11
rs18	1	3500	+	G	A	47	0.94	0.2765957	25	18
rs29	1	5750	-	G	T	45	0.90	0.1555556	32	12

	P.22	Pexact	Fmax	Plrt
rs10	1	1.0000000	0.02131603	0.8843626
rs18	4	0.7245853	0.04298643	0.7697067
rs29	1	1.0000000	-0.01503759	0.9188943

This shows summary of first three genotypes for people with age greater than or equal to 65 y.o. The same result may be achieved by sub-setting using a vector of logical values:

```
> vec <- (phdata(srdta)$age>=65)
> table(vec)
```

```
vec
FALSE  TRUE
2450    50
```

```
> summary(gtdata(srdta[vec,1:3]))
```



```

      Chromosome Position Strand A1 A2 NoMeasured CallRate      Q.2 P.11 P.12
rs10           1      2500      +   T   G           48      0.96 0.1354167   36   11
rs18           1      3500      +   G   A           47      0.94 0.2765957   25   18
rs29           1      5750      -   G   T           45      0.90 0.1555556   32   12
      P.22      Pexact      Fmax      Plrt
rs10      1 1.0000000 0.02131603 0.8843626
rs18      4 0.7245853 0.04298643 0.7697067
rs29      1 1.0000000 -0.01503759 0.9188943

```

or a list with IDs of study subjects:

```

> vec1 <- idnames(srdta)[vec]
> vec1

[1] "p64"   "p122"  "p186"  "p206"  "p207"  "p286"  "p385"  "p386"  "p492"
[10] "p514"  "p525"  "p536"  "p545"  "p565"  "p613"  "p632"  "p649"  "p673"
[19] "p701"  "p779"  "p799"  "p981"  "p1008" "p1131" "p1186" "p1223" "p1281"
[28] "p1383" "p1471" "p1489" "p1501" "p1565" "p1584" "p1673" "p1679" "p1782"
[37] "p1821" "p1832" "p1866" "p1891" "p1953" "p2081" "p2085" "p2140" "p2224"
[46] "p2268" "p2291" "p2384" "p2420" "p2453"

```

```

> summary(gtdata(srdta[vec1,1:3]))

```

```

      Chromosome Position Strand A1 A2 NoMeasured CallRate      Q.2 P.11 P.12
rs10           1      2500      +   T   G           48      0.96 0.1354167   36   11
rs18           1      3500      +   G   A           47      0.94 0.2765957   25   18
rs29           1      5750      -   G   T           45      0.90 0.1555556   32   12
      P.22      Pexact      Fmax      Plrt
rs10      1 1.0000000 0.02131603 0.8843626
rs18      4 0.7245853 0.04298643 0.7697067
rs29      1 1.0000000 -0.01503759 0.9188943

```

Let us explore the object returned by `summary` function when applied to `snp.data` class in more details:

```

> a <- summary(gtdata(srdta[vec1,1:3]))
> class(a)

```

```

[1] "data.frame"

```

Thus, the object returned is a `data.frame`. Therefore it should have dimensions and names:

```

> dim(a)

```

```

[1]  3 14

```

```

> names(a)

```

```

[1] "Chromosome" "Position"   "Strand"     "A1"         "A2"
[6] "NoMeasured" "CallRate"   "Q.2"        "P.11"       "P.12"
[11] "P.22"       "Pexact"     "Fmax"       "Plrt"

```

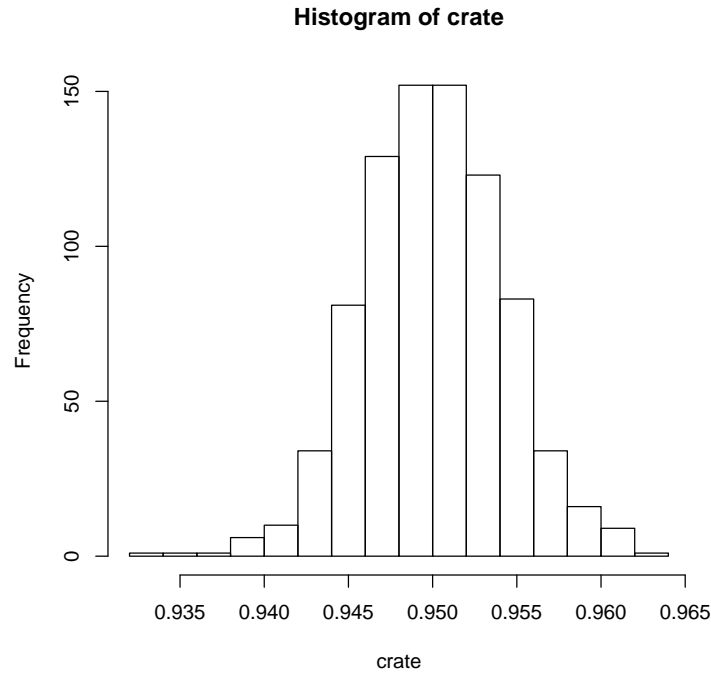


Figure 4.1: Histogram of the call rate

Indeed, we derived 8 characteristics ("NoMeasured", "CallRate", "Q.2", "P.11", "P.12", "P.22", "Pexact", "Chromosome") for the first 3 SNPs.

#### Exercise 4. Testing HWE for 10 SNPs

Test if Hardy-Weinberg equilibrium holds for the first 10 SNPs

1. Total sample
2. In cases (`bt` is 1)
3. In controls (`bt` is 0)

Let us analyse the distribution of call rate in the whole study. For this, we first need to obtain the vector of call rates:

```
> sumgt <- summary(gtdata(srdta))
> crate <- sumgt[, "CallRate"]
```

This vector may be presented by a histogram

```
> hist(crate)
```

which shows that most SNPs have call rate between 93 and 97% (figure 4.1).

As next step, you would like to produce a summary table, showing how many markers had call rate lower than, say, 93%, between 93 and 95%, between 95 and 99% and more than 99%. You can use `catable()` command for that:

```
> catable(crate,c(.93,.95,.99))
```

	X<=0.93	0.93<X<=0.95	0.95<X<=0.99	X>0.99
No	0	415.000	418.000	0
Prop	0	0.498	0.502	0

Similar procedure may be applied to see deviation from HWE:

```
> hwp <- sumgt[, "Pexact"]
> catable(hwp,c(0.05/nsnps(srdta),0.01,0.05,0.1))
```

	X<=6.00240096038415e-05	6.00240096038415e-05<X<=0.01	0.01<X<=0.05
No	2.000	7.000	23.000
Prop	0.002	0.008	0.028

	0.05<X<=0.1	X>0.1
No	31.000	770.000
Prop	0.037	0.924

The first cut-off category will detect SNPs which are deviating from HWE at the Bonferroni-corrected P-level.

However, for these data it will make more sense to table cumulative distribution:

```
> catable(hwp,c(0.05/nsnps(srdta),0.01,0.05,0.1),cum=T)
```

	X<=6.00240096038415e-05	X<=0.01	X<=0.05	X<=0.1	all X
No	2.000	9.000	32.000	63.000	833
Prop	0.002	0.011	0.038	0.076	1

If you would like to investigate the minor allele frequency (MAF) distribution, the same logic would apply. First, derive MAF with

```
> afr <- sumgt[, "Q.2"]
> maf <- pmin(afr,(1.-afr))
```

Next, generate histograms for frequency and MAF:

```
> par(mfcol=c(2,1))
> hist(afr)
> hist(maf)
```

(shown at the figure 4.2) and then generate table describing frequency distribution:

```
> catable(afr,c(0.01,0.05,0.1,0.2,0.5,0.8,0.9,0.95,0.99))
```

	X<=0.01	0.01<X<=0.05	0.05<X<=0.1	0.1<X<=0.2	0.2<X<=0.5	0.5<X<=0.8
No	22.000	53.000	99.000	132.000	313.000	187.000
Prop	0.026	0.064	0.119	0.158	0.376	0.224

	0.8<X<=0.9	0.9<X<=0.95	0.95<X<=0.99	X>0.99
No	18.000	8.00	1.000	0
Prop	0.022	0.01	0.001	0

```
> catable(maf,c(0,0.01,0.05,0.1,0.2),cum=T)
```

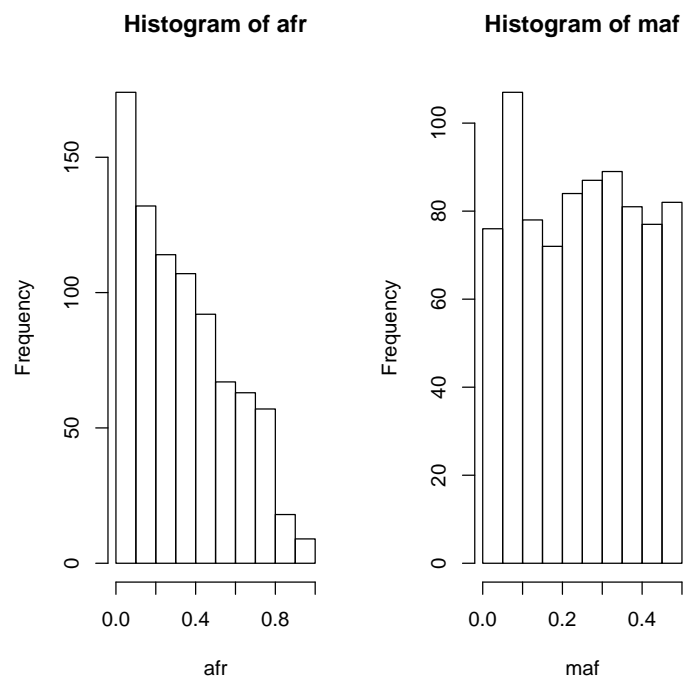


Figure 4.2: Histogram of the call rate

	X<=0	X<=0.01	X<=0.05	X<=0.1	X<=0.2	all X
No	0	22.000	76.000	183.00	333.0	833
Prop	0	0.026	0.091	0.22	0.4	1

Note that we used "0" as the first category – this will give you the number of monomorphic SNPs which we recommend to exclude from analysis.

Other function, `perid.summary`, produces summary SNP statistics per person. Let us try producing this summary for the first 10 people:

```
> perid.summary(srdta[1:10,])
```

	NoMeasured	NoPoly	Hom	E(Hom)	Var	F	CallPP
p1	790	707	0.7987342	0.6600319	0.4048662	0.407986159	0.9483794
p2	792	714	0.7474747	0.6585152	0.5090002	0.260508049	0.9507803
p3	783	700	0.6206897	0.6618209	0.4332890	-0.121625581	0.9399760
p4	789	705	0.6070976	0.6601276	0.5251900	-0.156029161	0.9471789
p5	790	707	0.6658228	0.6619821	0.5288936	0.011362319	0.9483794
p6	787	703	0.7662008	0.6622227	0.3770418	0.307830275	0.9447779
p7	794	709	0.6309824	0.6587669	0.4527349	-0.081423884	0.9531813
p8	793	711	0.7023960	0.6587232	0.5163296	0.127968868	0.9519808
p9	788	711	0.6675127	0.6573272	0.5599395	0.029723748	0.9459784
p10	797	713	0.6587202	0.6614644	0.4889042	-0.008105999	0.9567827

	Het
p1	0.2012658
p2	0.2525253
p3	0.3793103
p4	0.3929024
p5	0.3341772
p6	0.2337992
p7	0.3690176
p8	0.2976040
p9	0.3324873
p10	0.3412798

This table lists the number of genotypes scored for the person, call rate, and heterozygosity. The outliers who have increased average heterozygosity may be suggestive of contaminated DNA samples.

Let us analyse the distribution of heterozygosity:

```
> het <- perid.summary(srdta)$Het
> mean(het)

[1] 0.3309457

> catable(het,c(0.1,0.25,0.3,0.35,0.5))
```

	X<=0.1	0.1<X<=0.25	0.25<X<=0.3	0.3<X<=0.35	0.35<X<=0.5	X>0.5
No	7.000	73.000	339.000	1281.000	800.00	0
Prop	0.003	0.029	0.136	0.512	0.32	0

```
> hist(het)
```

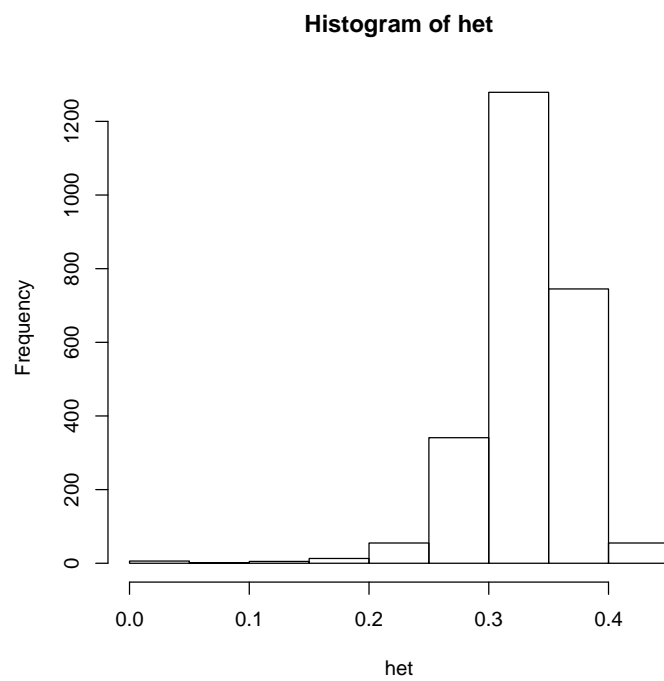


Figure 4.3: Histogram of heterozygosity

The resulting histogram is presented in figure 4.3. It is easy to see that few people have very low heterozygosity, but there are no outliers with extremely high values.

In this section, we covered low-level functions `summary` and `perid.summary`. Based on these, an upper-level genetic data quality control function, `check.marker`, is based. That function will be covered in the next section.

#### Summary:

- When `summary()` function is applied to an `gtdata` subset of `gwaa.data-class`, it return summary statistics for SNPs, including exact test for Hardy-Weinberg equilibrium.
- When `perid.summary()` function is applied to an object of `gwaa.data-class` (or `gtdata` part of it), it return per-person summary statistics, including the call rate within this person and its' heterozygosity.

#### Exercise 5. Characterizing call rate

Characterise the distribution of call rates within study subjects and produce a histogram. How many people have call rate below 93%?

## 4.5 Answers to exercises

**Answer (Ex. 1)** — Load the data with

```
> data(srdta)
```

Number of people:

```
> nids(srdta)
```

```
[1] 2500
```

Number of males:

```
> sum(male(srdta))
```

```
[1] 1275
```

Number of females:

```
> nids(srdta) - sum(male(srdta))
```

```
[1] 1225
```

... or you could get the same answer like this<sup>1</sup>:

```
> sum(male(srdta)==0)
```

```
[1] 1225
```

The proportion of males can be computed using above results

---

<sup>1</sup> This is something covered later in the section 4.3 ("Sub-setting and coercing `gwaa.data`")

```
> sum(male(srdta))/nids(srdta)
[1] 0.51
```

or by using `mean()` function:

```
> mean(male(srdta))
[1] 0.51
```

**Answer (Ex. 2)** — The names of markers located after 2,490,000 b.p. are

```
> vec <- (map(srdta)>2490000)
> snpnames(srdta)[vec]
[1] "rs9273" "rs9277" "rs9279" "rs9283"
```

The names of markers located between 1,100,000 and 1,105,000 b.p. are:

```
> vec <- (map(srdta)>1100000 & map(srdta)<1105000)
> snpnames(srdta)[vec]
[1] "rs4180" "rs4186" "rs4187"
```

**Answer (Ex. 3)** — To learn what allele of "rs114" is the reference you need to run

```
> coding(srdta)["rs114"]
rs114
"AT"
```

Here, the first ("A") allele is the reference and thus the second ("T") is the effective one. Remember that when using `as.numeric` function to convert the genotypes to human-readable and R-operatable format, the homozygotes for reference will be coded as "0", heterozygotes as "1" and the non-reference ("effective") homozygotes will be coded as "2":

```
> table(as.character(gtdata(srdta[, "rs114"])), as.numeric(gtdata(srdta[, "rs114"])))
```

	0	1	2
A/A 1868	0	0	
A/T	0	491	0
T/T	0	0	34

To compute frequency of the effective allele of SNP "rs114" in total sample, you can go two ways. First, we can try to take a sum of all "rs114" genotypes and divide it by twice the number of people:

```
> a <- as.numeric(gtdata(srdta[, "rs114"]))
> sum(a)
[1] NA
```

This, however, returns NA, because some of the genotypes are missing. We can deal with this problem by running `sum()` with the option `na.rm=TRUE`:

```
> sum(a, na.rm=T)
[1] 559
```



so the number of 'effect' alleles is 559.

However, now we do not know what was the number of people for whom the genotype was measured! – `nids` would return the *total* number of people, but not the number of ones measured for "rs114".

This problem can be dealt with through using `is.na(A)` function which returns true when some element of **A** is not measured. Thus, the number of people with measured genotype for "rs114" is

```
> nids(srdta)
[1] 2500
> nmeasured <- sum(!is.na(a))
> nmeasured
[1] 2393
```

(note the "!" before `is.na`, which means NOT, so we get these elements which are not NA). The frequency of the 'effect' allele thus is

```
> sum(a, na.rm=T)/(2*nmeasured)
[1] 0.116799
```

An easier way would be to compute mean value of "rs114" with the `mean( ... , na.rm=TRUE)` function and divide it by 2:

```
> mean(a, na.rm=T)/2
[1] 0.116799
```

To compute frequency of the effective allele of "rs114" in males, you can use

```
> amale <- as.numeric(gtdata(srdta[male(srdta)==1, "rs114"]))
> mean(amale, na.rm=T)/2
[1] 0.1164216
```

To compute frequency of the effective allele in females, you can use

```
> afemale <- as.numeric(gtdata(srdta[male(srdta)==0, "rs114"]))
> mean(afemale, na.rm=T)/2
[1] 0.1171942
```

The frequencies of the reference allele are computed very simply as one minus the frequency of the effective allele:

```
> 1 - mean(a, na.rm=T)/2
[1] 0.883201
> 1 - mean(amale, na.rm=T)/2
[1] 0.8835784
> 1 - mean(afemale, na.rm=T)/2
[1] 0.8828058
```

**Answer (Ex. 4)** — To test for HWE in first 10 SNPs in total sample

```
> summary(gtdata(srdta[, 1:10]))
```

	Chromosome	Position	Strand	A1	A2	NoMeasured	CallRate	Q.2	P.11	P.12
rs10	1	2500	+	T	G	2384	0.9536	0.13255034	1792	552
rs18	1	3500	+	G	A	2385	0.9540	0.28029350	1232	969
rs29	1	5750	-	G	T	2374	0.9496	0.13774221	1763	568
rs65	1	13500	+	A	T	2378	0.9512	0.71972246	182	969
rs73	1	14250	+	A	G	2385	0.9540	0.01341719	2331	44
rs114	1	24500	+	A	T	2393	0.9572	0.11679900	1868	491
rs128	1	27000	-	G	T	2391	0.9564	0.02488499	2281	101
rs130	1	27250	+	A	G	2379	0.9516	0.69377890	222	1013
rs143	1	31000	+	T	G	2377	0.9508	0.47728229	655	1175
rs150	1	33250	+	C	A	2369	0.9476	0.65998312	267	1077

	P.22	Pexact	Fmax	Plrt
rs10	40	7.897327e-01	-0.006880004	7.355343e-01
rs18	184	7.608230e-01	-0.007017332	7.315304e-01
rs29	43	7.955141e-01	-0.007241148	7.227853e-01
rs65	1227	6.475412e-01	-0.010016746	6.246577e-01
rs73	10	1.792470e-12	0.303150234	1.281239e-12
rs114	34	7.663683e-01	0.005487764	7.894076e-01
rs128	9	9.408599e-06	0.129600629	1.000431e-05
rs130	1144	9.615127e-01	-0.002140946	9.168114e-01
rs143	547	6.512540e-01	0.009313705	6.497695e-01
rs150	1025	5.518478e-01	-0.012948436	5.281254e-01

To test it in cases

```
> summary(gtdata(srdta[phdata(srdta)$bt==1,1:10]))
```

	Chromosome	Position	Strand	A1	A2	NoMeasured	CallRate	Q.2	P.11
rs10	1	2500	+	T	G	1197	0.9622186	0.13700919	888
rs18	1	3500	+	G	A	1189	0.9557878	0.28511354	605
rs29	1	5750	-	G	T	1176	0.9453376	0.14285714	859
rs65	1	13500	+	A	T	1185	0.9525723	0.72700422	83
rs73	1	14250	+	A	G	1187	0.9541801	0.01053075	1167
rs114	1	24500	+	A	T	1190	0.9565916	0.12184874	918
rs128	1	27000	-	G	T	1183	0.9509646	0.02409129	1129
rs130	1	27250	+	A	G	1188	0.9549839	0.68392256	117
rs143	1	31000	+	T	G	1192	0.9581994	0.48489933	320
rs150	1	33250	+	C	A	1182	0.9501608	0.66624365	127

	P.12	P.22	Pexact	Fmax	Plrt
rs10	290	19	4.635677e-01	-0.024514202	3.871421e-01
rs18	490	94	7.759191e-01	-0.010949158	7.052930e-01
rs29	298	19	2.832575e-01	-0.034722222	2.214580e-01
rs65	481	621	4.647357e-01	-0.022595469	4.348023e-01
rs73	15	5	3.988770e-08	0.393614304	2.423624e-08
rs114	254	18	8.924018e-01	0.002606831	9.285104e-01
rs128	51	3	2.747904e-02	0.083175674	3.157174e-02
rs130	517	554	8.407527e-01	-0.006569292	8.207476e-01
rs143	588	284	6.848365e-01	0.012522119	6.654994e-01
rs150	535	520	5.568363e-01	-0.017756050	5.409408e-01

in controls

```
> summary(gtdata(srdta[phdata(srdta)$bt==0,1:10]))
```

	Chromosome	Position	Strand	A1	A2	NoMeasured	CallRate	Q.2	P.11
rs10	1	2500	+	T	G	1177	0.9453815	0.12744265	897
rs18	1	3500	+	G	A	1185	0.9518072	0.27426160	623
rs29	1	5750	-	G	T	1188	0.9542169	0.13215488	897
rs65	1	13500	+	A	T	1183	0.9502008	0.71344041	98
rs73	1	14250	+	A	G	1188	0.9542169	0.01641414	1154
rs114	1	24500	+	A	T	1192	0.9574297	0.11157718	941
rs128	1	27000	-	G	T	1197	0.9614458	0.02589808	1141
rs130	1	27250	+	A	G	1181	0.9485944	0.70491109	104
rs143	1	31000	+	T	G	1174	0.9429719	0.46805792	334
rs150	1	33250	+	C	A	1176	0.9445783	0.65306122	139
	P.12	P.22	Pexact		Fmax		Plrt		
rs10	260	20	7.933317e-01	0.006751055	8.178295e-01				
rs18	474	88	9.418133e-01	-0.004812165	8.683219e-01				
rs29	268	23	5.288436e-01	0.016525913	5.737373e-01				
rs65	482	603	8.871139e-01	0.003540522	9.031273e-01				
rs73	29	5	6.941219e-06	0.244001185	5.537568e-06				
rs114	236	15	8.846527e-01	0.001356081	9.627084e-01				
rs128	50	6	7.745807e-05	0.172107564	7.552399e-05				
rs130	489	588	8.887439e-01	0.004728114	8.710047e-01				
rs143	581	259	8.604122e-01	0.006165442	8.326938e-01				
rs150	538	499	7.968462e-01	-0.009574142	7.424986e-01				

SNPs 'rs73' and 'rs128' are out of HWE (at  $p \leq 0.05$ ) in total sample, and also in cases and controls.

**Answer (Ex. 5)** — To characterize ID call rate, you can run the following commands:

```
> idsummary <- perid.summary(srdta)
> idsummary[1:5,]
      NoMeasured NoPoly      Hom      E(Hom)      Var      F      CallPP
p1          790      790 0.7987342 0.6696986 0.5448255 0.3906601 0.9483794
p2          792      792 0.7474747 0.6685502 0.5390602 0.2381191 0.9507803
p3          783      783 0.6206897 0.6712102 0.4888671 -0.1536561 0.9399760
p4          789      789 0.6070976 0.6700900 0.4077382 -0.1909382 0.9471789
p5          790      790 0.6658228 0.6710232 0.4340010 -0.0158077 0.9483794
      Het
p1 0.2012658
p2 0.2525253
p3 0.3793103
p4 0.3929024
p5 0.3341772
> idcall <- idsummary$Call
> idcall[1:5]
[1] 0.9483794 0.9507803 0.9399760 0.9471789 0.9483794
> catable(idcall,c(0.9,0.93,0.95,0.98,0.99))
      X<=0.9 0.9<X<=0.93 0.93<X<=0.95 0.95<X<=0.98 0.98<X<=0.99 X>0.99
No          0      13.000      1186.000      1301.00          0          0
Prop         0       0.005       0.474       0.52          0          0
```

```
> table(idcall<0.93)
```

```
FALSE  TRUE  
2487    13
```

To produce a histogram of call rates, use `hist(idcall)`

## Chapter 5

# Genome-wide association analysis

In the first parts of this section you will be guided through a GWA analysis of a small data set. In the last part you will investigate a larger data set by yourself, do a verification study and will answer the questions. All data sets used assume a study in a relatively homogeneous population. Try to finish the first part in the morning and the second part in the afternoon.

Though only few thousands of markers located at four small chromosomes are used in the scan, we still going to call it Genome-Wide (GW), as the amount of data we will use is approaches the amount to be expected in a real experiment. However, because the regions are small, and the LD between SNPs is high, some specific features (e.g. relatively high residual inflation, which occurs because large proportion of SNPs are in LD with the reuly associated ones) are specific features of this data set, which are not observed in true GWA studies.

Start R and load **GenABEL**-package library by typing

```
> library(GenABEL)
```

and load the data which we will use in this section by

```
> data(ge03d2ex)
```

Investigate the objects loaded by command

```
> ls()
```

```
[1] "ge03d2ex" "old"
```

The `ge03d2ex` is an object of the class `gwaa.data`:

```
> class(ge03d2ex)
```

```
[1] "gwaa.data"
attr(,"package")
[1] "GenABEL"
```

To check what are the names of variables in the phenotypic data frame, use

```
> names(phdata(ge03d2ex))
```

```
[1] "id"      "sex"      "age"      "dm2"      "height" "weight" "diet"      "bmi"
```

We can attach this data frame to the R search path by

```
> attach(phdata(ge03d2ex))
```

## 5.1 Data descriptives and first round of GWA analysis

Let us investigate what are the traits presented in the data frame loaded and what are the characteristics of the distribution by using specific **GenABEL**-package function `descriptive.trait`:

```
> descriptives.trait(ge03d2ex)
```

	No	Mean	SD
id	136	NA	NA
sex	136	0.529	0.501
age	136	49.069	12.926
dm2	136	0.632	0.484
height	135	169.440	9.814
weight	135	87.397	25.510
diet	136	0.059	0.236
bmi	135	30.301	8.082

You can see that phenotypic frame contains the data on 136 people; the data on sex, age, height, weight, diet and body mass index (BMI) are available. Our trait of interest is `dm2` (type 2 diabetes). Note that every single piece of information in this data set is simulated; however, we tried to keep our simulations in a way we think the control of T2D may work.

You can produce a summary for cases and controls separately and compare distributions of the traits by

```
> descriptives.trait(ge03d2ex,by=dm2)
```

	No(by.var=0)	Mean	SD	No(by.var=1)	Mean	SD	Ptt	Pkw
id	50	NA	NA	86	NA	NA	NA	NA
sex	50	0.420	0.499	86	0.593	0.494	0.053	0.052
age	50	47.038	13.971	86	50.250	12.206	0.179	0.205
dm2	50	NA	NA	86	NA	NA	NA	NA
height	49	167.671	8.586	86	170.448	10.362	0.097	0.141
weight	49	76.534	17.441	86	93.587	27.337	0.000	0.000
diet	50	0.060	0.240	86	0.058	0.235	0.965	0.965
bmi	49	27.304	6.463	86	32.008	8.441	0.000	0.001

	Pexact
id	NA
sex	0.074
age	NA
dm2	NA

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```
height      NA
weight      NA
diet        1.000
bmi         NA
```

Here, the `by` argument specifies the grouping variable. You can see that cases and controls are different in weight, which is expected, as T2D is associated with obesity.

Similarly, you can produce grand GW descriptives of the marker data by using

```
> descriptives.marker(ge03d2ex)

$`Minor allele frequency distribution`
      X<=0.01 0.01<X<=0.05 0.05<X<=0.1 0.1<X<=0.2 X>0.2
No    146.000      684.000      711.000      904.000 1555.000
Prop   0.036       0.171       0.178       0.226   0.389

$`Cumulative distr. of number of SNPs out of HWE, at different alpha`
      X<=1e-04 X<=0.001 X<=0.01 X<=0.05 all X
No       46.000    71.000 125.000 275.000 4000
Prop     0.012     0.018  0.031  0.069    1

$`Distribution of proportion of successful genotypes (per person)`
      X<=0.9 0.9<X<=0.95 0.95<X<=0.98 0.98<X<=0.99 X>0.99
No       1.000          0          0       135.000      0
Prop    0.007          0          0       0.993      0

$`Distribution of proportion of successful genotypes (per SNP)`
      X<=0.9 0.9<X<=0.95 0.95<X<=0.98 0.98<X<=0.99 X>0.99
No      37.000      6.000     996.000     1177.000 1784.000
Prop    0.009      0.002      0.249      0.294   0.446

$`Mean heterozygosity for a SNP`
[1] 0.2582298

$`Standard deviation of the mean heterozygosity for a SNP`
[1] 0.1592255

$`Mean heterozygosity for a person`
[1] 0.2476507

$`Standard deviation of mean heterozygosity for a person`
[1] 0.04291038
```

It is of note that we can see inflation of the proportion of the tests for HWE at particular threshold, as compared to the expected. This may indicate poor genotyping quality and/or genetic stratification.

We can test the GW marker characteristics in controls by

```
> descriptives.marker(ge03d2ex,ids=(dm2==0))
```

```

$`Minor allele frequency distribution`
      X<=0.01 0.01<X<=0.05 0.05<X<=0.1 0.1<X<=0.2  X>0.2
No    233.000      676.000      671.000      898.000 1522.00
Prop   0.058        0.169        0.168        0.224  0.38

$`Cumulative distr. of number of SNPs out of HWE, at different alpha`
      X<=1e-04 X<=0.001 X<=0.01 X<=0.05 all X
No           0      3.000  14.000  98.000  4000
Prop          0      0.001  0.003  0.024    1

$`Distribution of proportion of successful genotypes (per person)`
      X<=0.9 0.9<X<=0.95 0.95<X<=0.98 0.98<X<=0.99 X>0.99
No          0           0           0           50      0
Prop         0           0           0           1      0

$`Distribution of proportion of successful genotypes (per SNP)`
      X<=0.9 0.9<X<=0.95 0.95<X<=0.98 0.98<X<=0.99 X>0.99
No    37.000      49.000      1523.000           0 2391.000
Prop  0.009      0.012      0.381           0  0.598

$`Mean heterozygosity for a SNP`
[1] 0.2555009

$`Standard deviation of the mean heterozygosity for a SNP`
[1] 0.1618707

$`Mean heterozygosity for a person`
[1] 0.252572

$`Standard deviation of mean heterozygosity for a person`
[1] 0.04714886

```

Apparently, HWE distribution holds better in controls than in the total sample.

Let us check whether there are indications that deviation from HWE is due to cases. At this stage we are only interested in HWE distribution table, and therefore will ask to report the distribution for cases (`dm2==1`) and report only the table two:

```

> descriptives.marker(ge03d2ex,ids=(dm2==1))[2]

$`Cumulative distr. of number of SNPs out of HWE, at different alpha`
      X<=1e-04 X<=0.001 X<=0.01 X<=0.05 all X
No      45.000      79.00 136.000 268.000  4000
Prop    0.011      0.02  0.034  0.067    1

and for the controls

> descriptives.marker(ge03d2ex,ids=(dm2==0))[2]

$`Cumulative distr. of number of SNPs out of HWE, at different alpha`
      X<=1e-04 X<=0.001 X<=0.01 X<=0.05 all X
No           0      3.000  14.000  98.000  4000
Prop          0      0.001  0.003  0.024    1

```



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It seems that indeed excessive number of markers are out of HWE in cases. If no laboratory procedure (e.g. DNA extraction, genotyping, calling) were done for cases and controls separately, this may indicate possible genetic heterogeneity specific for cases.

In essence, `'descriptives.marker'` function uses `'summary'` function to generate HW P-values distribution. It may be interesting to generate this distribution using the `'summary'` function. You do not need to do so, but this example shows how you can generate summaries from underlying SNP-tables. First, we need to compute summary SNP statistics by

```
> s <- summary(gtdata(ge03d2ex[(dm2==1),]))
```

Note that you have produced the summary for the `gtdata` slot of `ge03d2ex`; this is the slot which actually contains all genetic data in special compressed format.

You can see first 5 rows of this very long summary table by

```
> s[1:5,]
```

	Chromosome	Position	Strand	A1	A2	NoMeasured	CallRate	Q.2	P.11
rs1646456	1	653	+	C	G	85	0.9883721	0.32941176	34
rs4435802	1	5291	+	C	A	86	1.0000000	0.09302326	70
rs946364	1	8533	-	T	C	84	0.9767442	0.24404762	46
rs299251	1	10737	+	A	G	85	0.9883721	0.03529412	79
rs2456488	1	11779	+	G	C	85	0.9883721	0.33529412	38
	P.12	P.22	Pexact	Fmax	Plrt				
rs1646456	46	5	0.05089075	-0.22493734	0.03233812				
rs4435802	16	0	1.00000000	-0.10256410	0.19978917				
rs946364	35	3	0.37413083	-0.12924909	0.21777250				
rs299251	6	0	1.00000000	-0.03658537	0.63937447				
rs2456488	37	10	0.81059718	0.02344356	0.82918345				

Note that the column `'Pexact'` provides exact HWE test *P*-values we need. We can extract these to a separate vector by

```
> pexcas <- s[, "Pexact"]
```

and do characterization of the cumulative distribution by

```
> catable(pexcas, c(0.001, 0.01, 0.05, 0.1, 0.5), cumulative=TRUE)
```

	X<=0.001	X<=0.01	X<=0.05	X<=0.1	X<=0.5	all X
No	79.00	136.000	268.000	390.000	1359.00	4000
Prop	0.02	0.034	0.067	0.098	0.34	1

You can generate the distribution for controls in similar manner.

Let us first try to do GWA scan using the raw (before quality control) data. We will use the score test, as implemented in the `qtscore()`<sup>1</sup> function of `GenABEL`-package for testing:

```
> an0 <- qtscore(dm2, ge03d2ex, trait="binomial")
```

<sup>1</sup>consider `'mlreg'` function if you want to run true linear regression

The first argument used describes the model; here it is rather simple — the affection status, `dm2`, is supposed to depend on SNP genotype only.

You can see what information is computed by this function by using

```
> an0

***** 'scan.gwaa' object *****
*** Produced with:
qtscore(formula = dm2, data = ge03d2ex, trait.type = "binomial")
*** Test used: binomial
*** no. IDs used: 136 ( id199 id287 id300 , ... )
*** Lambda: 1.033102
*** Results table contains 4000 rows and 9 columns
*** Output for 10 first rows is:
      N      effB      se_effB      chi2.1df      P1df      effAB      effBB
rs1646456 135 0.9487666  5.0501959 0.0352941176 0.8509807 1.5558824 0.4831933
rs4435802 134 2.6822601  1.6765631 2.5595403237 0.1096305 2.5142857      NA
rs946364  134 0.6376645  0.4012104 2.5260391714 0.1119810 0.7277883 0.2869565
rs299251  135 0.5592122  0.5740215 0.9490674319 0.3299568 0.5569620      NA
rs2456488 135 0.8669860  1.5393112 0.3172278551 0.5732784 0.9736842 0.6907895
rs3712159 133 0.8282737  2.4803134 0.1115153279 0.7384255 0.5641026      Inf
rs4602970 136 1.5227297  2.2683336 0.4506421219 0.5020302 1.5131579      NA
rs175910  134 0.9949826 57.3970087 0.0003005055 0.9861693 0.5600000 4.0727273
rs1919938 136 0.9303079  3.5515057 0.0686164762 0.7933619 0.1160000 0.1958333
rs8892781 133 1.0953022 14.9055712 0.0053997133 0.9414220 1.0952381      NA
      chi2.2df      P2df
rs1646456 3.885667042 0.14329734
rs4435802 2.559540324 0.10963046
rs946364  3.020970009 0.22080286
rs299251  0.949067432 0.32995679
rs2456488 0.493411146 0.78137072
rs3712159 1.358996877 0.50687116
rs4602970 0.450642122 0.50203018
rs175910  5.012993241 0.08155345
rs1919938 6.001763944 0.04974318
rs8892781 0.005399713 0.94142198
...
___ Use 'results(object)' to get complete results table ___
```

Here, let us look at the 'Results table'. `P1df`, `P2df` and `Pc1df` are most interesting; the first two are vectors of 1 and 2 d.f. *P*-values obtained in the GWA analysis, the last one is 1 d.f. *P*-value corrected for inflation factor  $\lambda$  (which is presented in `lambda` object). `effB` corresponds to (approximate) Odds Ratios estimate for the SNP.

Let us see if there is evidence for the inflation of the test statistics; for that let us obtain  $\lambda$  with

```
> lambda(an0)

$estimate
[1] 1.033102
```

```
$se
[1] 0.0005639231
```

```
$iz0
[1] 1
```

```
$iz2
[1] 1
```

The estimate of  $\lambda$  is 1.03, suggesting inflation of the test and some degree of stratification. Though the value obtained seems to be small, it should be noted that  $\lambda$  grows linearly with sample size, so for this small number of cases and controls the value is worrisome.

The  $\lambda$  is computed by regression in a Q-Q plot. Both estimation of  $\lambda$  and production of the  $\chi^2 - \chi^2$  plot can be done using the `estlambda` function; this was already done automatically when running `qtscore` function, but let us repeat this manually:

```
> estlambda(an0[, "P1df"], plot=TRUE)
```

```
$estimate
[1] 1.033102
```

```
$se
[1] 0.0005639231
```

The corresponding  $\chi^2 - \chi^2$  plot is presented in Figure 5.1.

**The 'se' produced by `estlambda` *can not* be used to test if inflation is significant and make conclusions about presence of significant or insignificant stratification.**

We can also present the obtained results using the "Manhattan plot", where a SNP genomic position is on the X-axes and  $-\log_{10}$  of the  $P$ -value is shown on Y-axes:

```
> plot(an0)
```

The resulting plot is presented in the figure 5.2. By default,  $-\log_{10}(P\text{-value})$  of not corrected 1 d.f. test are presented; see help to figure out how this behaviour can be changed.

We can also add the corrected  $P$ -values to the plot with

```
> add.plot(an0, df="Pc1df", col=c("lightblue", "lightgreen"))
```

You can see that  $P$ -values corrected by genomic control are uniformly lower than the  $P$ -values from 'raw' analysis. This is to be expected as genomic control simply divides the 'raw'  $\chi^2$  statistics by a constant  $\lambda$  for all SNPs.

You can also generate a descriptive table for the "top" (as ranked by  $P$ -value) results by

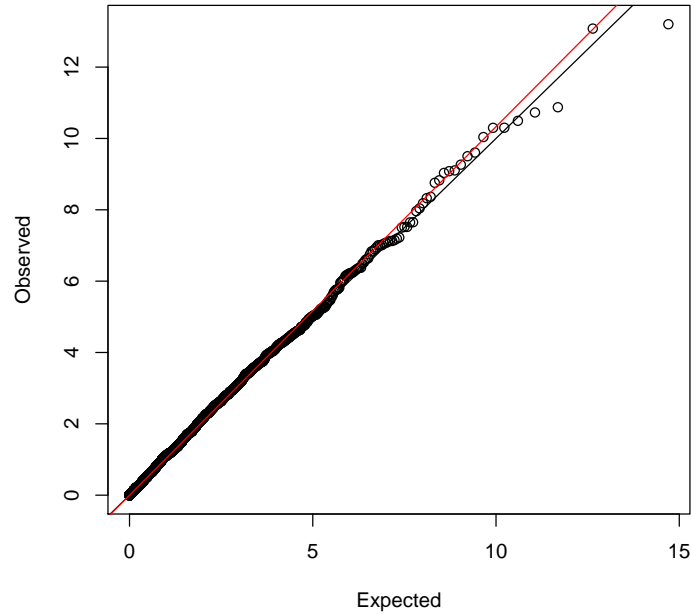


Figure 5.1:  $\chi^2 - \chi^2$  plot for a GWA scan. Black line of slope 1: expected under no inflation; Red line: fitted slope.

```
> descriptives.scan(an0)
```

Summary for top 10 results, sorted by P1df

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB	chi2.1df
rs1719133	1	4495479	+	T	A	136	0.33729339	0.09282784	13.202591
rs2975760	3	10518480	+	A	T	134	3.80380024	1.05172986	13.080580
rs7418878	1	2808520	+	A	T	136	3.08123060	0.93431795	10.875745
rs5308595	3	10543128	-	C	G	133	3.98254950	1.21582875	10.729452
rs4804634	1	2807417	+	C	G	132	0.43411456	0.13400290	10.494949
rs3224311	2	6009769	+	G	C	135	3.15831710	0.98401491	10.301681
rs26325	3	10617781	+	A	C	135	0.09742793	0.03035964	10.298495
rs8835506	2	6010852	+	A	T	132	3.17720829	1.00274087	10.039543
rs3925525	2	6008501	+	C	G	135	2.98416931	0.96286458	9.605423
rs2521089	3	10487652	-	T	C	135	2.50239493	0.81179595	9.502064
	P1df	effAB		effBB	chi2.2df		P2df	Pc1df	
rs1719133	0.0002795623	0.4004237		0.000000	14.729116		0.0006333052	0.0003504258	
rs2975760	0.0002983731	3.4545455	10.000000		13.547345		0.0011434877	0.0003732694	
rs7418878	0.0009743183	3.6051282	4.871795		12.181064		0.0022642036	0.0011762545	
rs5308595	0.0010544366	3.3171429		Inf	10.766439		0.0045930101	0.0012699705	
rs4804634	0.0011970132	0.5240642	0.173913		11.200767		0.0036964462	0.0014362332	
rs3224311	0.0013290907	3.4151786	4.250000		11.658283		0.0029405999	0.0015897278	
rs26325	0.0013313876	0.1097724		NA	10.298495		0.0013313876	0.0015923930	

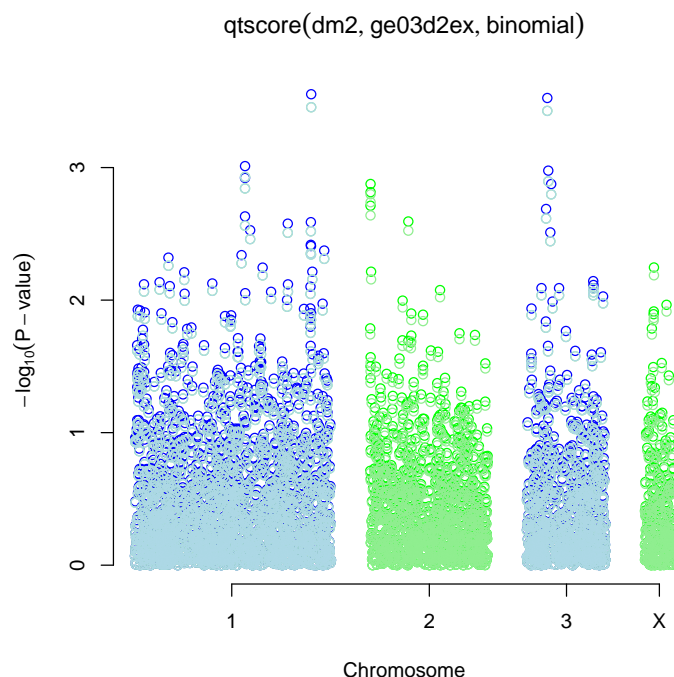


Figure 5.2:  $-\log_{10}(P\text{-value})$  from the genome scan before QC procedure. Raw analysis: darker circles; corrected analysis: lighter circles

```
rs8835506 0.0015321522 3.4903846 4.125000 11.513206 0.0031618340 0.0018248521
rs3925525 0.0019400358 3.2380952 4.121212 10.782867 0.0045554384 0.0022944719
rs2521089 0.0020524092 2.5717703 4.772727 9.933387 0.0069661425 0.0024233145
```

or, equivalently, by `'summary(an0)'`

Here you see top 10 results, sorted by P-value with 1 d.f. If you want to sort by the corrected P-value, you can use `descriptives.scan(an0,sort="Pc1df")`; to see more than 10 (e.g. 25) top results, use `descriptives.scan(an0,top=25)`. You can combine all these options. Large part of results reports NA as effect estimates and 9.99 as *P-value* for 2 d.f. test – for these markers only two out of three possible genotypes were observed, and consequently 2 d.f. test could not be performed.

Now let us apply `qtscore()` function with `times` argument, which tells it to compute empirical GW (or experiment-wise) significance

```
> an0.e <- qtscore(dm2,ge03d2ex,times=200,quiet=TRUE)
```

```
|
|
|
|=====| 100%
```

(you may skip the `'quiet=TRUE'` argument, then you will see progress)

Now let us generate the summary of the results

```
> descriptives.scan(an0.e,sort="Pc1df")
```

Summary for top 10 results, sorted by Pc1df

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB	chi2.1df
rs1719133	1	4495479	+	T	A	136	-0.2652064	0.07298850	13.202591
rs2975760	3	10518480	+	A	T	134	0.2340655	0.06471782	13.080580
rs7418878	1	2808520	+	A	T	136	0.2089098	0.06334746	10.875745
rs5308595	3	10543128	-	C	G	133	0.2445516	0.07465893	10.729452
rs4804634	1	2807417	+	C	G	132	-0.2050449	0.06329344	10.494949
rs3224311	2	6009769	+	G	C	135	0.2133633	0.06647611	10.301681
rs26325	3	10617781	+	A	C	135	-0.4875367	0.15192190	10.298495
rs8835506	2	6010852	+	A	T	132	0.2112000	0.06665565	10.039543
rs3925525	2	6008501	+	C	G	135	0.2057095	0.06637371	9.605423
rs2521089	3	10487652	-	T	C	135	0.1775016	0.05758287	9.502064
	P1df	Pc1df	effAB	effBB	chi2.2df	P2df			
rs1719133	0.385	0.485	-0.2080882	-0.7375000	14.729116	NA			
rs2975760	0.415	0.510	0.2755102	0.4090909	13.547345	NA			
rs7418878	0.855	0.915	0.2807405	0.3268398	12.181064	NA			
rs5308595	0.880	0.935	0.2564832	0.4623656	10.766439	NA			
rs4804634	0.920	0.950	-0.1193830	-0.3845238	11.200767	NA			
rs3224311	0.940	0.965	0.2778634	0.3151515	11.658283	NA			
rs26325	0.940	0.965	-0.4875367	NA	10.298495	NA			
rs8835506	0.955	0.975	0.2796221	0.3076923	11.513206	NA			
rs3925525	0.985	0.995	0.2660834	0.3074627	10.782867	NA			
rs2521089	0.990	0.995	0.2254633	0.3396072	9.933387	NA			

Experimental-wise significance is computed by the empirical procedure, thus we consider  $P$ -values  $\leq 0.05$  to be GW-significant. However, none of the SNPs hits GW significance. If, actually, any did pass the threshold, we could not trust the results, because the distribution of the HWE test and presence of inflation factor for the association test statistics suggest that the data may contain multiple errors (indeed they do). Therefore before association analysis we need to do rigorous Quality Control (QC).

Note that at certain SNP, corrected  $P$ -values become equal to 1 – at this point order is arbitrary because sorting could not be done.

#### Summary:

- The `descriptives` family of functions was developed to facilitate production of tables which can be directly used in a manuscript — it is possible to save the output as a file, which can be open by Excel or Word. See e.g. `help(descriptives.trait)` for details.
- The inflation of test statistics compared to null (1 d.f.) may be estimated with `estlambda` function.

## 5.2 Genetic data QC

The major genetic data QC function of GenABEL-package is `check.marker()`. We will now use that to perform our data QC; the output is rather self-explaining. Because of possible genetic heterogeneity of the study data it is good idea to skip Hardy-Weinberg checks in the first round of QC. This can be achieved by setting HWE P-value selection threshold to zero (`p.level=0`):

```
> qc1 <- check.marker(ge03d2ex,p.level=0)
```

Excluding people/markers with extremely low call rate...

4000 markers and 136 people in total

0 people excluded because of call rate < 0.1

6 markers excluded because of call rate < 0.1

Passed: 3994 markers and 136 people

Running sex chromosome checks...

197 heterozygous X-linked male genotypes found

1 X-linked markers are likely to be autosomal (odds > 1000 )

2 male are likely to be female (odds > 1000 )

0 female are likely to be male (odds > 1000 )

0 people have intermediate X-chromosome inbreeding ( $0.5 > F > 0.5$ )

If these people/markers are removed, 0 heterozygous male genotypes are left

Passed: 3993 markers and 134 people

no X/Y/mtDNA-errors to fix

RUN 1

3993 markers and 134 people in total

304 (7.613323%) markers excluded as having low (<1.865672%) minor allele frequency

36 (0.9015778%) markers excluded because of low (<95%) call rate

0 (0%) markers excluded because they are out of HWE ( $P < 0$ )

1 (0.7462687%) people excluded because of low (<95%) call rate

Mean autosomal HET is 0.2747262 (s.e. 0.03721277)

3 (2.238806%) people excluded because too high autosomal heterozygosity (FDR <1%)

Excluded people had HET  $\geq$  0.4856887

Mean IBS is 0.7687408 (s.e. 0.02044218), as based on 2000 autosomal markers

2 (1.492537%) people excluded because of too high IBS ( $\geq 0.95$ )

In total, 3653 (91.4851%) markers passed all criteria

In total, 128 (95.52239%) people passed all criteria

RUN 2

3653 markers and 128 people in total

80 (2.189981%) markers excluded as having low (<1.953125%) minor allele frequency

0 (0%) markers excluded because of low (<95%) call rate

0 (0%) markers excluded because they are out of HWE ( $P < 0$ )

0 (0%) people excluded because of low (<95%) call rate

Mean autosomal HET is 0.2748341 (s.e. 0.01695461)

0 people excluded because too high autosomal heterozygosity (FDR <1%)

Mean IBS is 0.7714623 (s.e. 0.01871215), as based on 2000 autosomal markers

```

0 (0%) people excluded because of too high IBS (>=0.95)
In total, 3573 (97.81002%) markers passed all criteria
In total, 128 (100%) people passed all criteria

RUN 3
3573 markers and 128 people in total
0 (0%) markers excluded as having low (<1.953125%) minor allele frequency
0 (0%) markers excluded because of low (<95%) call rate
0 (0%) markers excluded because they are out of HWE (P <0)
0 (0%) people excluded because of low (<95%) call rate
Mean autosomal HET is 0.2748341 (s.e. 0.01695461)
0 people excluded because too high autosomal heterozygosity (FDR <1%)
Mean IBS is 0.7725624 (s.e. 0.01783144), as based on 2000 autosomal markers
0 (0%) people excluded because of too high IBS (>=0.95)
In total, 3573 (100%) markers passed all criteria
In total, 128 (100%) people passed all criteria

```

Note that normally you will *NEVER* run this simple form of the QC function – you should always provide a number of thresholds specific to the platform you used for genotyping. See help to `check.marker()` for detailed list of arguments. Default values used by the function are rather relaxed compared to the thresholds routinely used nowadays with most of the platforms.

**The computation of all pairwise proportion of alleles identical-by-state (IBS) by `ibs()` function, which is also called by `check.marker()` may take quite some time, which is proportional to the square of the number of subjects. This is not a problem with the small number of people we use for this example or when modern computers are used. However, the computers in the Nihes computer room are very old. Therefore be prepared to wait for long time when you will do a self-exercise with 1,000 people.**

From the output you can see that QC starts with checking the data for SNPs and people with extremely low call rate. Six markers are excluded from further analysis due to very low call rate. Next, X-chromosomal errors are identified. The function finds out that all errors (heterozygous male X-genotypes) are due to two people with wrong sex assigned and one marker, which looks like an autosomal one. This actually could be a marker from pseudoautosomal region, which should have been arranged as a separate "autosome". Nine people are found to have intermediate inbreeding at the X-chromosome and also excluded from analysis.

Then, the procedure finds the markers with low call rate ( $\leq 0.95$  by default) across people, markers with low MAF (by default, low MAF is defined as less than few copies of the rare allele, see help for details); people with low call rate (default value:  $\leq 0.95$ ) across SNPs, people with extreme heterozygosity (at FDR 0.01) and these who have GW IBS  $\geq 0.95$ . These default parameters may be changed if you wish (consult help).

Because some of the people fail to pass the tests, the data set is not guaranteed to be really "clean" after single iteration, e.g. some marker may not



pass the call threshold after we exclude few informative (but apparently having low quality) samples. Therefore the QC is repeated iteratively until no further errors are found.

You can generate short summary of QC by marker and by person through

```
> summary(qc1)
```

```
$`Per-SNP fails statistics`
```

	NoCall	NoMAF	NoHWE	Redundant	Xsnpfail
NoCall	42	0	0	0	0
NoMAF	NA	384	0	0	0
NoHWE	NA	NA	0	0	0
Redundant	NA	NA	NA	0	0
Xsnpfail	NA	NA	NA	NA	1

```
$`Per-person fails statistics`
```

	IDnoCall	HetFail	IBSFail	isfemale	ismale	isXXY	otherSexErr
IDnoCall	1	0	0	0	0	0	0
HetFail	NA	3	0	0	0	0	0
IBSFail	NA	NA	2	0	0	0	0
isfemale	NA	NA	NA	2	0	0	0
ismale	NA	NA	NA	NA	0	0	0
isXXY	NA	NA	NA	NA	NA	0	0
otherSexErr	NA	NA	NA	NA	NA	NA	0

Note that the original data, `ge03d2ex`, are not modified during the procedure; rather, `check.markers()` generate a list of markers and people which pass or do not pass certain QC criteria. The objects returned by `check.markers()` are:

```
> names(qc1)
```

```
[1] "nofreq"      "nocall"      "nohwe"      "Xmrkfail"    "hetfail"
[6] "idnocall"    "ibsfail"     "isfemale"    "ismale"      "otherSexErr"
[11] "snpok"      "idok"        "call"
```

The element `idok` provides the list of people who passed all QC criteria, and `snpok` provides the list of SNPs which passed all criteria. You can easily generate a new data set, which will consist only of these people and markers by

```
> data1 <- ge03d2ex[qc1$idok,qc1$snpok]
```

If there are any residual sporadic X-errors (male heterozygosity), these can (and should!) be fixed (set to NA) by

```
> data1 <- Xfix(data1)
```

```
no X/Y/mtDNA-errors to fix
```

Applying this function does not make any difference for the example data set, but you will need to use it for the bigger data set.

At this point, we are ready to work with the new, cleaned, data set `data1`. However, if we try

```
> length(dm2)
```

```
[1] 136
```

we can see that the original phenotypic data are attached to the search path (there are only 128 people left in the 'clean' data set). Therefore we need to detach the data by

```
> detach(phdata(ge03d2ex))
```

and attach new data by

```
> attach(phdata(data1))
```

At this stage, let us check if the first round of QC improves the fit of genetic data to HWE, which may have been violated due to by genotyping errors which we hopefully (at least partly!) eliminated:

```
> descriptives.marker(data1)[2]
```

```
$`Cumulative distr. of number of SNPs out of HWE, at different alpha`
      X<=1e-04 X<=0.001 X<=0.01 X<=0.05 all X
No      44.000   66.000 117.000 239.000 3573
Prop    0.012    0.018  0.033  0.067    1
```

```
> descriptives.marker(data1[dm2==1,])[2]
```

```
$`Cumulative distr. of number of SNPs out of HWE, at different alpha`
      X<=1e-04 X<=0.001 X<=0.01 X<=0.05 all X
No      46.000   70.00 127.000 228.000 3573
Prop    0.013    0.02  0.036  0.064    1
```

```
> descriptives.marker(data1[dm2==0,])[2]
```

```
$`Cumulative distr. of number of SNPs out of HWE, at different alpha`
      X<=1e-04 X<=0.001 X<=0.01 X<=0.05 all X
No           0         0   7.000  91.000 3573
Prop         0         0  0.002  0.025    1
```

You can see that the fit to HWE improved, but cases are still have excess number of markers out of HWE. This may be due to genetic sub-structure.

### 5.3 Finding genetic sub-structure

Now, we are ready for the second round of QC – detection of genetic outliers which may contaminate our results. We will detect genetic outliers using a technique, which resembles the one suggested by Price et al.

As the first step, we will compute a matrix of genomic kinship between all pairs of people, using only autosomal<sup>2</sup> markers by

```
> data1.gkin <- ibs(data1[,autosomal(data1)],weight="freq")
```

---

<sup>2</sup>the list of autosomal markers contained in `data` is returned by `autosomla(data)` function

This step may take few minutes on large data sets or when using old computers!

You can see the 5x5 upper left sub-matrix by

```
> data1.gkin[1:5,1:5]
```

	id199	id300	id403	id415	id666
id199	0.494427766	3255.00000000	3253.00000000	3241.00000000	3257.00000000
id300	-0.011754266	0.49360296	3261.00000000	3250.00000000	3264.00000000
id403	-0.012253378	-0.01262949	0.50541775	3247.00000000	3262.00000000
id415	-0.001812109	0.01388179	-0.02515438	0.53008236	3251.00000000
id666	-0.018745051	-0.02127344	0.02083723	-0.02014175	0.5306584

The numbers below the diagonal show the genomic estimate of kinship (aka 'genomic kinship' or 'genome-wide IBD'), the numbers on the diagonal correspond to 0.5 plus the genomic homozygosity, and the numbers above the diagonal tell how many SNPs were typed successfully for both subjects (thus the IBD estimate is derived using this number of SNPs).

Second, we transform this matrix to a distance matrix using standard R command

```
> data1.dist <- as.dist(0.5-data1.gkin)
```

Finally, we perform Classical Multidimensional Scaling by

```
> data1.mds <- cmdscale(data1.dist)
```

by default, the first two principal components are computed and returned.

This may take few minutes on large data sets or when using old computers!

We can present the results graphically by

```
> plot(data1.mds)
```

The resulting plot is presented in figure 5.3. Each point on the plot corresponds to a person, and the 2D distances between points were fitted to be as close as possible to these presented in the original IBS matrix. You can see that study subjects clearly cluster in two groups.

You can identify the points belonging to clusters by

```
> km <- kmeans(data1.mds,centers=2,nstart=1000)
> c11 <- names(which(km$cluster==1))
> c12 <- names(which(km$cluster==2))
> if (length(c11)>length(c12)) {x<-c12;c12<-c11;c11<-x}
> c11
```

[1] "id2097" "id6954" "id2136" "id858"

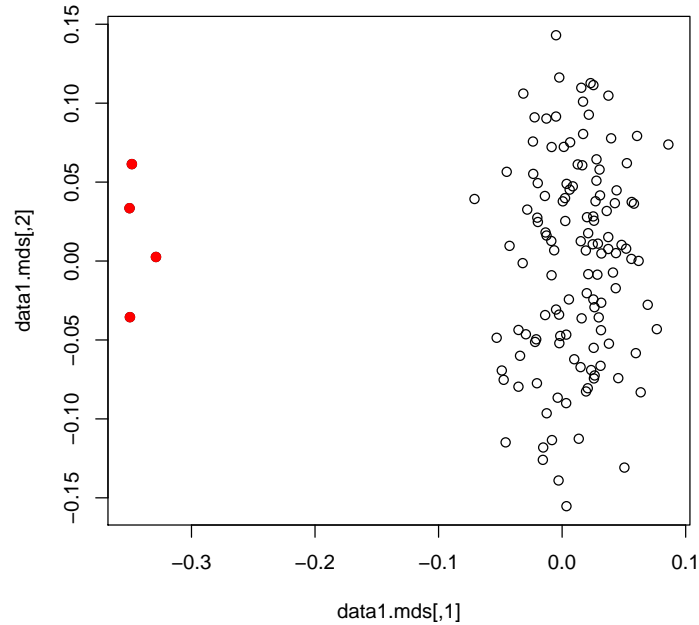


Figure 5.3: Mapping samples on the space of the first two Principle Components resulting from analysis of genomic kinship. Red dots identify genetic outliers

```
> c12
```

```
[1] "id199" "id300" "id403" "id415" "id666" "id689" "id765" "id830"
[9] "id908" "id980" "id994" "id1193" "id1423" "id1505" "id1737" "id1827"
[17] "id1841" "id2068" "id2094" "id2151" "id2317" "id2618" "id2842" "id2894"
[25] "id2985" "id3354" "id3368" "id3641" "id3831" "id3983" "id4097" "id4328"
[33] "id4380" "id4395" "id4512" "id4552" "id4710" "id4717" "id4883" "id4904"
[41] "id4934" "id4961" "id5014" "id5078" "id5274" "id5275" "id5454" "id5853"
[49] "id5926" "id5969" "id6237" "id6278" "id6352" "id6501" "id6554" "id6663"
[57] "id6723" "id7499" "id7514" "id7541" "id7598" "id7623" "id7949" "id8059"
[65] "id8128" "id8281" "id8370" "id8400" "id8433" "id8772" "id8880" "id8890"
[73] "id8957" "id8996" "id9082" "id9901" "id9930" "id1857" "id2528" "id4862"
[81] "id9184" "id5677" "id6407" "id5472" "id2135" "id8545" "id4333" "id1670"
[89] "id1536" "id6917" "id6424" "id3917" "id9628" "id9635" "id4729" "id5190"
[97] "id6399" "id6062" "id620" "id1116" "id6486" "id41" "id677" "id4947"
[105] "id9749" "id6428" "id7488" "id5949" "id2924" "id5783" "id4096" "id903"
[113] "id9049" "id185" "id1002" "id362" "id9014" "id5044" "id2749" "id2286"
[121] "id4743" "id4185" "id8330" "id6934"
```

Four outliers are presented in the smaller cluster.

Now you will need to use the BIGGER cluster for to select study subjects.  
Whether this will be c11 or c12 in you case, is totally random.

We can form a data set which is free from outliers by using only people from the bigger cluster:

```
> data2 <- data1[c12,]
```

After we dropped the outliers, we need to repeat QC using `check.markers()`. At this stage, we want to allow for HWE checks (we will use only controls and exclude markers with  $FDR \leq 0.2$ ):

```
> qc2 <- check.marker(data2,hweids=(phdata(data2)$dm2==0),fdr=0.2)
```

Excluding people/markers with extremely low call rate...

```
3573 markers and 124 people in total
0 people excluded because of call rate < 0.1
0 markers excluded because of call rate < 0.1
Passed: 3573 markers and 124 people
```

Running sex chromosome checks...

```
0 heterozygous X-linked male genotypes found
0 X-linked markers are likely to be autosomal (odds > 1000 )
0 male are likely to be female (odds > 1000 )
0 female are likely to be male (odds > 1000 )
0 people have intermediate X-chromosome inbreeding (0.5 > F > 0.5)
If these people/markers are removed, 0 heterozygous male genotypes are left
Passed: 3573 markers and 124 people
```

no X/Y/mtDNA-errors to fix

RUN 1

```
3573 markers and 124 people in total
40 (1.119507%) markers excluded as having low (<2.016129%) minor allele frequency
0 (0%) markers excluded because of low (<95%) call rate
0 (0%) markers excluded because they are out of HWE (FDR <0.2)
0 (0%) people excluded because of low (<95%) call rate
Mean autosomal HET is 0.2780246 (s.e. 0.01642372)
0 people excluded because too high autosomal heterozygosity (FDR <1%)
Mean IBS is 0.7692747 (s.e. 0.01251359), as based on 2000 autosomal markers
0 (0%) people excluded because of too high IBS (>=0.95)
In total, 3533 (98.88049%) markers passed all criteria
In total, 124 (100%) people passed all criteria
```

RUN 2

```
3533 markers and 124 people in total
0 (0%) markers excluded as having low (<2.016129%) minor allele frequency
0 (0%) markers excluded because of low (<95%) call rate
```

```

0 (0%) markers excluded because they are out of HWE (FDR <0.2)
0 (0%) people excluded because of low (<95%) call rate
Mean autosomal HET is 0.2780246 (s.e. 0.01642372)
0 people excluded because too high autosomal heterozygosity (FDR <1%)
Mean IBS is 0.7715673 (s.e. 0.01240002), as based on 2000 autosomal markers
0 (0%) people excluded because of too high IBS (>=0.95)
In total, 3533 (100%) markers passed all criteria
In total, 124 (100%) people passed all criteria

```

```
> summary(qc2)
```

```
$`Per-SNP fails statistics`
```

	NoCall	NoMAF	NoHWE	Redundant	Xsnpfail
NoCall	0	0	0	0	0
NoMAF	NA	40	0	0	0
NoHWE	NA	NA	0	0	0
Redundant	NA	NA	NA	0	0
Xsnpfail	NA	NA	NA	NA	0

```
$`Per-person fails statistics`
```

	IDnoCall	HetFail	IBSFail	isfemale	ismale	isXXY	otherSexErr
IDnoCall	0	0	0	0	0	0	0
HetFail	NA	0	0	0	0	0	0
IBSFail	NA	NA	0	0	0	0	0
isfemale	NA	NA	NA	0	0	0	0
ismale	NA	NA	NA	NA	0	0	0
isXXY	NA	NA	NA	NA	NA	0	0
otherSexErr	NA	NA	NA	NA	NA	NA	0

If the procedure did not run, check previous Note.

Indeed, in the updated data set few markers do not pass our QC criteria and we need to drop a few markers. This is done by

```
> data2 <- data2[qc2$idok,qc2$snpok]
```

This is going to be our final analysis data set, therefore let us attach the phenotypic data to the search path, then we do not need to type `phdata(data2)$...` to access `dm2` status or other variables:

```

> detach(phdata(data1))
> attach(phdata(data2))
> ####
> #ge03d2ex.clean <- data2
> #save(ge03d2ex.clean,file="ge03d2ex.clean.RData")
> ####

```

Before proceeding to GWA, let us check if complete QC improved the fit of genetic data to HWE:

```
> descriptives.marker(data2)[2]

$`Cumulative distr. of number of SNPs out of HWE, at different alpha`
      X<=1e-04 X<=0.001 X<=0.01 X<=0.05 all X
No          1      2.000    2e+01 101.000 3533
Prop         0      0.001    6e-03  0.029    1

> descriptives.marker(data2[phdata(data2)$dm2==1,])[2]

$`Cumulative distr. of number of SNPs out of HWE, at different alpha`
      X<=1e-04 X<=0.001 X<=0.01 X<=0.05 all X
No           0          1  17.000  79.000 3533
Prop         0          0   0.005   0.022    1

> descriptives.marker(data2[phdata(data2)$dm2==0,])[2]

$`Cumulative distr. of number of SNPs out of HWE, at different alpha`
      X<=1e-04 X<=0.001 X<=0.01 X<=0.05 all X
No           0          0   7.000  91.000 3533
Prop         0          0   0.002   0.026    1
```

You can see that now there is no excessive number of SNPs out of HWE in the sample (total, or cases, or controls)

## 5.4 GWA association analysis

Let us start again with descriptives of the phenotypic and marker data

```
> descriptives.trait(data2,by=dm2)
```

	No(by.var=0)	Mean	SD	No(by.var=1)	Mean	SD	Ptt	Pkw
id	47	NA	NA	77	NA	NA	NA	NA
sex	47	0.426	0.500	77	0.597	0.494	0.065	0.064
age	47	45.752	13.313	77	50.593	12.465	0.047	0.062
dm2	47	NA	NA	77	NA	NA	NA	NA
height	46	167.911	8.689	77	170.423	10.646	0.157	0.213
weight	46	77.015	17.528	77	94.160	26.963	0.000	0.000
diet	47	0.064	0.247	77	0.065	0.248	0.981	0.981
bmi	46	27.424	6.598	77	32.235	8.335	0.001	0.001

	Pexact
id	NA
sex	0.067
age	NA
dm2	NA
height	NA
weight	NA
diet	1.000
bmi	NA

You can see that relation to weight is maintained in this smaller, but hopefully cleaner, data set; moreover, relation to age becomes boundary significant.

If you check descriptives of markers (only HWE part shown)

```
> descriptives.marker(data2)[2]
```

```
$`Cumulative distr. of number of SNPs out of HWE, at different alpha`
      X<=1e-04 X<=0.001 X<=0.01 X<=0.05 all X
No          1      2.000    2e+01 101.000  3533
Prop         0      0.001    6e-03   0.029    1
```

you can see that the problems with HWE are apparently fixed; we may guess that these were caused by the Wahlund's effect.

Run the score test on the cleaned data by

```
> data2.qt <- qtscore(dm2,data2,trait="binomial")
```

and check lambda

```
> lambda(data2.qt)
```

```
$estimate
[1] 1.036958
```

```
$se
[1] 0.0007178
```

```
$iz0
[1] 1.009061
```

```
$iz2
[1] 1
```

there is still some inflation, which is explained by the fact that we investigate only few short chromosomes with high LD and few causative variants.

Produce the association analysis plot by

```
> plot(data2.qt,df="Pc1df")
```

(figure 5.4).

Produce the scan summary by

```
> descriptives.scan(data2.qt,sort="Pc1df")
```

Summary for top 10 results, sorted by Pc1df

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB	chi2.1df
rs1719133	1	4495479	+	T	A	124	0.3167801	0.08614528	13.522368
rs4804634	1	2807417	+	C	G	121	0.4119844	0.12480696	10.896423
rs8835506	2	6010852	+	A	T	121	3.5378209	1.08954331	10.543448
rs4534929	1	4474374	+	C	G	123	0.4547151	0.14160410	10.311626
rs1013473	1	4487262	+	A	T	124	2.7839368	0.86860745	10.272393
rs3925525	2	6008501	+	C	G	124	3.2807631	1.03380675	10.070964
rs3224311	2	6009769	+	G	C	124	3.2807631	1.03380675	10.070964
rs2975760	3	10518480	+	A	T	123	3.1802120	1.00916993	9.930784
rs2521089	3	10487652	-	T	C	123	2.7298775	0.87761175	9.675679
rs1048031	1	4485591	+	G	T	122	0.4510793	0.14548378	9.613391
	P1df	effAB		effBB	chi2.2df		P2df		Pc1df



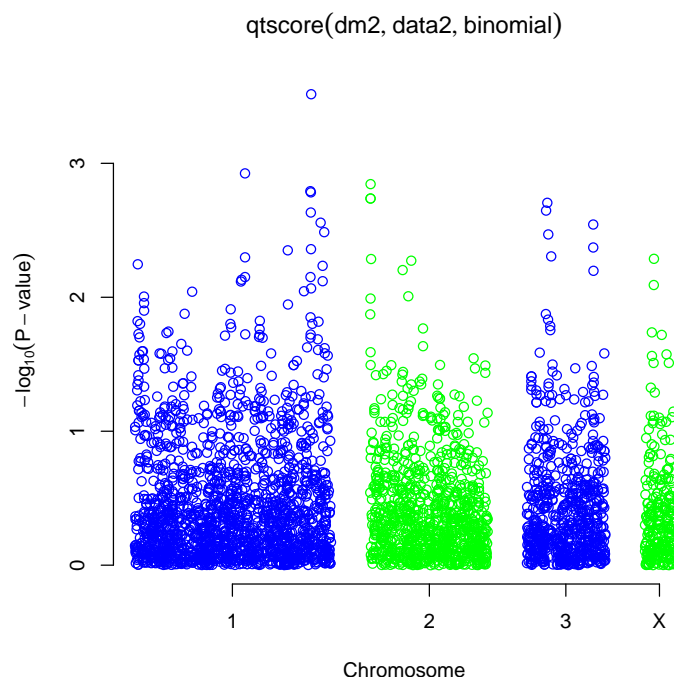


Figure 5.4:  $-\log_{10}(\text{Corrected } P\text{-value})$  from the genome scan after the QC procedure

```
rs1719133 0.0002357368 0.3740771 0.0000000 14.677906 0.0006497303 0.0003048399
rs4804634 0.0009635013 0.6315789 0.1739130 12.375590 0.0020543516 0.0011885463
rs8835506 0.0011660066 4.0185185 4.0185185 12.605556 0.0018312105 0.0014292471
rs4534929 0.0013219476 0.4830918 0.1739130 10.510272 0.0052206352 0.0016136479
rs1013473 0.0013503553 3.0495868 5.8441558 10.926296 0.0042401869 0.0016471605
rs3925525 0.0015062424 3.6923077 4.0000000 11.765985 0.0027864347 0.0018306610
rs3224311 0.0015062424 3.6923077 4.0000000 11.765985 0.0027864347 0.0018306610
rs2975760 0.0016253728 3.0000000 8.0000000 10.172522 0.0061810866 0.0019704699
rs2521089 0.0018672326 3.0147059 5.0000000 10.543296 0.0051351403 0.0022533033
rs1048031 0.0019316360 0.4844720 0.1714286 9.965696 0.0068545128 0.0023284084
```

Comparison with the top 10 from the scan before QC shows that results changed substantially with only few markers overlapping.

You can see similar results when accessing empirical GW significance:

```
> data2.qte <- qtscore(dm2,data2,times=200,quiet=TRUE,trait="binomial")

|
|
|
|=====| 100%

> descriptives.scan(data2.qte,sort="Pc1df")
```

Summary for top 10 results, sorted by Pc1df

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB	chi2.1df
rs1719133	1	4495479	+	T	A	124	0.3167801	0.08614528	13.5223679
rs4804634	1	2807417	+	C	G	121	0.4119844	0.12480696	10.8964232
rs8835506	2	6010852	+	A	T	121	3.5378209	1.08954331	10.5434485
rs4534929	1	4474374	+	C	G	123	0.4547151	0.14160410	10.3116260
rs1013473	1	4487262	+	A	T	124	2.7839368	0.86860745	10.2723932
rs3925525	2	6008501	+	C	G	124	3.2807631	1.03380675	10.0709636
rs3224311	2	6009769	+	G	C	124	3.2807631	1.03380675	10.0709636
rs2975760	3	10518480	+	A	T	123	3.1802120	1.00916993	9.9307842
rs2521089	3	10487652	-	T	C	123	2.7298775	0.87761175	9.6756789
rs1646456	1	653	+	C	G	123	0.8999026	2.43077426	0.1370572
	P1df	Pc1df	effAB	effBB	chi2.2df	P2df			
rs1719133	0.340	0.400	0.3740771	0.000000	14.677906	0.540			
rs4804634	0.845	0.915	0.6315789	0.173913	12.375590	0.930			
rs8835506	0.900	0.960	4.0185185	4.018519	12.605556	0.900			
rs4534929	0.940	0.975	0.4830918	0.173913	10.510272	1.000			
rs1013473	0.940	0.975	3.0495868	5.844156	10.926296	0.995			
rs3925525	0.965	0.985	3.6923077	4.000000	11.765985	0.975			
rs3224311	0.965	0.985	3.6923077	4.000000	11.765985	0.975			
rs2975760	0.975	0.990	3.0000000	8.000000	10.172522	1.000			
rs2521089	0.990	0.995	3.0147059	5.000000	10.543296	1.000			
rs1646456	1.000	1.000	1.4261460	0.483871	3.063906	1.000			

Again, none of the SNPs hits GW 5% significance. Still, you can see that after QC top markers achieve somewhat "better" significance.

In the last part, we will do several adjusted and stratified analyses. Only empirical P-values will be estimated to make the story shorter. To adjust for sex and age, we can

```
> data2.qtae <- qtscore(dm2~sex+age,data2,times=200,quiet=T,trait="binomial")

|
|
|
|=====| 100%

> descriptives.scan(data2.qtae)
```

Summary for top 10 results, sorted by P1df

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB	chi2.1df
rs1719133	1	4495479	+	T	A	124	0.5057834	0.1386464	13.307976
rs2398949	1	4828375	-	A	C	122	0.4163599	0.1253217	11.037861
rs4804634	1	2807417	+	C	G	121	0.5811444	0.1769232	10.789433
rs7522488	3	11689797	-	G	A	123	1.8718644	0.5707656	10.755565
rs3925525	2	6008501	+	C	G	124	2.0202686	0.6406356	9.944799
rs3224311	2	6009769	+	G	C	124	2.0202686	0.6406356	9.944799
rs8835506	2	6010852	+	A	T	121	2.0575431	0.6501899	10.014228
rs1037237	3	11690145	+	C	G	124	1.8144282	0.5735660	10.007206
rs1013473	1	4487262	+	A	T	124	1.8148181	0.5864622	9.576043
rs2939190	1	71843	-	A	G	124	0.5820057	0.1965457	8.768541

	P1df	Pc1df	effAB	effBB	chi2.2df	P2df
rs1719133	0.470	0.585	0.4853657	0.2242383	13.367455	0.835
rs2398949	0.825	0.930	0.3110042	2.8197228	16.111010	0.395
rs4804634	0.870	0.950	0.7420448	0.3389525	12.112794	0.980
rs7522488	0.875	0.955	1.3995557	3.1160361	11.256933	0.995
rs3925525	0.975	0.995	2.3028971	2.2451502	11.988964	0.985
rs3224311	0.975	0.995	2.3028971	2.2451502	11.988964	0.985
rs8835506	0.975	0.995	2.3758119	2.2365358	12.302956	0.965
rs1037237	0.975	0.995	1.3400938	2.9836475	10.674189	1.000
rs1013473	0.990	0.995	1.8390955	2.8613308	9.893424	1.000
rs2939190	0.995	1.000	0.4730825	0.4471353	9.841144	1.000

You can see that there is little difference between adjusted and unadjusted analysis, but this is not always the case; adjustment may make your study much more powerful when covariates explain a large proportion of environmental trait variation.

Finally, let us do stratified (by BMI) analysis. We will contract obese ( $BMI \geq 30$ ) cases to all controls.

```
> data2.qtse <- qtscore(dm2~sex+age,data2,ids=((bmi>30 & dm2==1) | dm2==0),times=200,quiet=TRUE)
```

```
|
|
|
|=====| 100%
```

```
> descriptives.scan(data2.qtse,sort="Pc1df")
```

Summary for top 10 results, sorted by Pc1df

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB	chi2.1df
rs7522488	3	11689797	-	G	A	88	1.7561171	0.5560485	9.9742822
rs1037237	3	11690145	+	C	G	88	1.7561171	0.5560485	9.9742822
rs9630764	1	3897972	+	T	A	88	1.7595598	0.5615384	9.8185891
rs1891586	1	2297398	-	C	T	88	0.5340528	0.1733886	9.4869655
rs794264	1	2534738	-	C	A	87	1.7252112	0.5832358	8.7497608
rs3215698	X	13559835	-	T	A	88	0.4806715	0.1571512	9.3553776
rs2884479	X	13618173	+	A	C	86	0.5896309	0.1990222	8.7772281
rs1646456	1	653	+	C	G	87	0.8853536	1.3981894	0.4009605
rs4435802	1	5291	+	C	A	86	1.7961795	1.1926370	2.2682082
rs946364	1	8533	-	T	C	86	0.7682453	0.5994013	1.6427231

	P1df	Pc1df	effAB	effBB	chi2.2df	P2df
rs7522488	0.925	0.940	1.3215457	3.1245402	11.299048	0.995
rs1037237	0.925	0.940	1.3215457	3.1245402	11.299048	0.995
rs9630764	0.955	0.980	1.9346110	3.3161548	9.831616	1.000
rs1891586	0.985	0.985	0.5836292	0.3065275	9.518213	1.000
rs794264	0.995	0.995	2.2543966	3.5369544	8.965654	1.000
rs3215698	0.985	0.995	0.3406693	0.2701336	9.941713	1.000
rs2884479	0.995	0.995	0.2872964	0.4639136	14.266996	0.580
rs1646456	1.000	1.000	1.1455730	0.6013127	2.201546	1.000
rs4435802	1.000	1.000	1.7919867	NA	2.268208	1.000
rs946364	1.000	1.000	0.8791195	0.4696496	2.188118	1.000

Again, noting interesting at GW significance level. If we would have had found something, naturally, we would not know if we mapped a T2D or obesity gene (or a gene for obesity in presence of T2D, or the one for T2D in presence of obesity).

Let us save 'data1', 'data1.gkin', 'data2.qt' and 'cl1' objects now

```
> save(data1, cl1, data1.gkin, data2.qt, file="data1.RData")
```

These data will be used later in section 7, "GWA in presence of genetic stratification: practice", in which we will perform GWA using different methods to account for stratification, and will compare the results with those obtained by removing outliers ('data2.qt').

Let us also save the cleaned 'data2' object, which will be later used in section 10 ("Meta-analysis of GWA scans"):

```
> save(data2, file="data2.RData")
```

At this point, you acquired the knowledge necessary for the self-exercise. Please close R by `q()` command and proceed to the next section.

## 5.5 Genome-wide association analysis exercise

During the exercise, you will work with a larger data set (approximately 1,000 people and 7,000+ SNPs). You are to do complete three-round QC; perform GWA analysis with `dm2` as the outcome of interest and identify 10 SNPs which you would like to take to the stage 2 (replication) scan. You will do replication analysis using a confirmatory data set. If you did everything right, the SNPs which you identified as significant or replicated will be located in known T2D genes.

Please keep in mind that the data are simulated, and do not take your findings too seriously!

Start R by going to "Start -> Programs -> R -> R-?.?.?". Load **GenABEL**-package library by

```
> library(GenABEL)
```

The two data sets we will use in this exercise are part of the **GenABEL**-package distribution. The first one ("discovery" set) can be loaded by

```
> data(ge03d2)
```

Please move along the lines detailed in the guided exercise and try to answer following questions:

**Ex. 1** — How many cases and controls are presented in the original data set?

**Ex. 2** — How many markers are presented in the original data set?

**Ex. 3** — Is there evidence for inflation of the HWE test statistics?

**Ex. 4** — Analyse empirical GW significance. How many SNPs pass genome-wide significance threshold, after correction for the inflation factor? Write down the names of these SNPs for further comparison.

**Ex. 5** — Perform first steps of the genetic data QC.

**Ex. 6** — How many males are 'genetically' females?

**Ex. 7** — How many females are 'genetically' males?

**Ex. 8** — How many people are guessed to have 'XXY' genotype?

**Ex. 9** — How many sporadic X errors do you still observe even when the female male and non-X X-markers are removed? (do not forget to `Xfix()` these

**Ex. 10** — How many "twin" DNAs did you discover?

**Ex. 11** — Perform second step of QC.

**Ex. 12** — How many genetic outliers did you discover?

**Ex. 13** — How many cases and controls are presented in the data after QC?

**Ex. 14** — How many markers are presented in the data after QC?

**Ex. 15** — Is there evidence for inflation of the HWE test staistics?

**Ex. 16** — Perform GWA analysis of the cleaned data, using asimptotic test and plot the results. What is the estimate of  $\lambda$  for the 1 d.f. test?

**Ex. 17** — Analyse empirical GW significance. How many SNPs pass genome-wide significance threshold, after correction for the inflation factor?

**Ex. 18** — Do these SNPs overlap much with the ones ranked at the top before the QC? If not, what could be the reason?

**Ex. 19** — Select 10 SNPs which you would like to follow-up. Say, you've selected rs1646456, rs7950586, rs4785242, rs4435802, rs2847446, rs946364, rs299251, rs2456488, rs1292700, and rs8183220. Make a vector of these SNPs with

```
> vec12<-c("rs1646456","rs7950586","rs4785242","rs4435802","rs2847446","rs946364","rs299251","r
```

Load the stage 2 (replicaton) data set by

```
> data(ge03d2c)
```

and select the subset of SNPs you need by

```
> confdat <- ge03d2c[,vec12]
```

Analyse the `confdat` for association with `dm2`.

**Ex. 20** — Given the two-stage design, and applying the puristic criteria specified in the lecture, for how many SNPs you can claim a significant finding?

**Ex. 21** — Using the same criteria, for how many SNPs you can claim a replicated finding?

**Ex. 22** — If time permits, characterise the mode of inheritance of the significant SNPs. You can convert data from `GenABEL`-package format to the format used by `dgc.genetics` and `genetics` libraries by using `as.genotype()` function. Consult help for details. Please do not attempt to convert more then few

dozens SNPs: the format of `genetics` is not compressed, which means conversion may take long and your low-memory computer may even crash if you attempt to convert the whole data set.

**Ex. 23** — If time permits, do analysis with adjustment for covariates and stratified analysis.

**Ex. 24** — If time permits, try to do first round of QC allowing for HWE checks (assume FDR of 0.1 for total sample). In this case, can you still detect stratification in the "cleaned" data?

## 5.6 Answers to exercises

**Answer (Ex. 1)** — :

```
> table(phdata(ge03d2)$dm2)
  0    1
487 463
```

**Answer (Ex. 2)** — :

```
> nsnpns(ge03d2)
[1] 7589
```

**Answer (Ex. 3)** — Yes:

```
> descriptives.marker(ge03d2)[2]
$`Cumulative distr. of number of SNPs out of HWE, at different alpha`
      X<=1e-04 X<=0.001 X<=0.01 X<=0.05 all X
No      331.000  367.000 479.000 807.000  7589
Prop    0.044    0.048   0.063   0.106    1
```

**Answer (Ex. 4)** — :

```
> res0 <- qtscore(dm2,data=ge03d2,times=200,quiet=TRUE,trait="binomial")
|
|
|
|=====| 100%
> lambda(res0)
$estimate
[1] 2.036652
$se
[1] 0.006781986
```

```

$iz0
[1] 1.665047

$iz2
[1] 2.569015

> ds <- descriptives.scan(res0)
Summary for top 10 results, sorted by P1df
> ds

```

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB	chi2.1df
rs2689086	1	47084	+	G	A	937	2.205520	0.44739635	24.30173
rs4950080	1	214792	+	C	A	941	2.484678	0.49819026	24.87423
rs6386070	1	583935	+	G	C	942	2.976929	0.57274468	27.01561
rs7539209	1	794073	+	T	A	941	2.661572	0.54458925	23.88572
rs7903146	1	1047389	+	A	T	940	0.482230	0.09228697	27.30409
rs9350372	1	1191492	-	C	G	937	2.267829	0.44434642	26.04818
rs3977642	1	1362820	+	G	A	944	2.451399	0.49878344	24.15483
rs3880649	1	1463316	-	T	C	940	2.775569	0.55366968	25.13058
rs6581033	1	1725546	-	G	C	940	2.572408	0.52822674	23.71587
rs3845221	1	1874765	+	G	T	942	3.249560	0.59849064	29.48047

```


```

	P1df	Pc1df	effAB	effBB	chi2.2df	P2df
rs2689086	0.004975124	0.885	1.5532286	29.1230366	27.91257	NA
rs4950080	0.004975124	0.840	1.4396285	Inf	28.55184	NA
rs6386070	0.004975124	0.655	1.1212121	Inf	28.27535	NA
rs7539209	0.004975124	0.920	1.1229314	Inf	27.25887	NA
rs7903146	0.004975124	0.635	0.5149630	0.1466667	27.54275	NA
rs9350372	0.004975124	0.770	1.5360195	Inf	31.03145	NA
rs3977642	0.004975124	0.890	1.6145833	28.0797101	25.95722	NA
rs3880649	0.004975124	0.830	0.9330986	Inf	28.27379	NA
rs6581033	0.004975124	0.930	1.0238095	Inf	28.38434	NA
rs3845221	0.004975124	0.450	4.5433255	Inf	29.56317	NA

```


```

Thus, there are no genome-wide empirically significant results. The 'top' 10 SNPs are

```

> snps0 <- rownames(ds)
> snps0
[1] "rs2689086" "rs4950080" "rs6386070" "rs7539209" "rs7903146" "rs9350372"
[7] "rs3977642" "rs3880649" "rs6581033" "rs3845221"

```

(note that if empirical P is 1, the rank is assigned quite arbitrarily)

### Answer (Ex. 5) — First step of QC

```

> qc1 <- check.marker(ge03d2,call=0.95,perid.call=0.95,p.level=0,ibs.exclude="both")
Excluding people/markers with extremely low call rate...
7589 markers and 950 people in total
0 people excluded because of call rate < 0.1
7 markers excluded because of call rate < 0.1
Passed: 7582 markers and 950 people

```

```

Running sex chromosome checks...
1934 heterozygous X-linked male genotypes found
2 X-linked markers are likely to be autosomal (odds > 1000 )
10 male are likely to be female (odds > 1000 )
6 female are likely to be male (odds > 1000 )
0 people have intermediate X-chromosome inbreeding (0.5 > F > 0.5)
If these people/markers are removed, 8 heterozygous male genotypes are left
... these will be considered missing in analysis.
... Use Xfix() to fix these problems.
Passed: 7580 markers and 934 people

... 8 X/Y/mtDNA ( 8 0 0 ) impossible heterozygotes and female Ys set as missing

```

```

RUN 1
7580 markers and 934 people in total
73 (0.9630607%) markers excluded as having low (<0.267666%) minor allele frequency
75 (0.9894459%) markers excluded because of low (<95%) call rate
0 (0%) markers excluded because they are out of HWE (P <0)
4 (0.4282655%) people excluded because of low (<95%) call rate
Mean autosomal HET is 0.2558271 (s.e. 0.02102863)
4 (0.4282655%) people excluded because too high autosomal heterozygosity (FDR <1%)
Excluded people had HET >= 0.4702949
Mean IBS is 0.7862282 (s.e. 0.01659049), as based on 2000 autosomal markers
8 (0.856531%) people excluded because of too high IBS (>=0.95)
In total, 7432 (98.04749%) markers passed all criteria
In total, 918 (98.28694%) people passed all criteria

```

```

RUN 2
7432 markers and 918 people in total
42 (0.5651238%) markers excluded as having low (<0.2723312%) minor allele frequency
0 (0%) markers excluded because of low (<95%) call rate
0 (0%) markers excluded because they are out of HWE (P <0)
0 (0%) people excluded because of low (<95%) call rate
Mean autosomal HET is 0.2562716 (s.e. 0.0151377)
0 people excluded because too high autosomal heterozygosity (FDR <1%)
Mean IBS is 0.7846618 (s.e. 0.01588122), as based on 2000 autosomal markers
0 (0%) people excluded because of too high IBS (>=0.95)
In total, 7390 (99.43488%) markers passed all criteria
In total, 918 (100%) people passed all criteria

```

```

RUN 3
7390 markers and 918 people in total
0 (0%) markers excluded as having low (<0.2723312%) minor allele frequency
0 (0%) markers excluded because of low (<95%) call rate
0 (0%) markers excluded because they are out of HWE (P <0)
0 (0%) people excluded because of low (<95%) call rate
Mean autosomal HET is 0.2562716 (s.e. 0.0151377)
0 people excluded because too high autosomal heterozygosity (FDR <1%)
Mean IBS is 0.7820564 (s.e. 0.01582289), as based on 2000 autosomal markers

```



0 (0%) people excluded because of too high IBS ( $\geq 0.95$ )

In total, 7390 (100%) markers passed all criteria

In total, 918 (100%) people passed all criteria

```
> summary(qc1)
```

```
$`Per-SNP fails statistics`
```

	NoCall	NoMAF	NoHWE	Redundant	Xsnpfail
NoCall	82	0	0	0	0
NoMAF	NA	115	0	0	0
NoHWE	NA	NA	0	0	0
Redundant	NA	NA	NA	0	0
Xsnpfail	NA	NA	NA	NA	2

```
$`Per-person fails statistics`
```

	IDnoCall	HetFail	IBSFail	isfemale	ismale	isXXY	otherSexErr
IDnoCall	4	0	0	0	0	0	0
HetFail	NA	4	0	0	0	0	0
IBSFail	NA	NA	8	0	0	0	0
isfemale	NA	NA	NA	10	0	0	0
ismale	NA	NA	NA	NA	6	0	0
isXXY	NA	NA	NA	NA	NA	0	0
otherSexErr	NA	NA	NA	NA	NA	NA	0

```
> data1 <- ge03d2[qc1$idok,qc1$snpok]
```

```
> data1 <- Xfix(data1)
```

```
... 7 X/Y/mtDNA ( 7 0 0 ) impossible heterozygotes and female Ys set as missing
```

```
> qc2 <- check.marker(data1,call=0.95,perid.call=0.95,p.level=0)
```

Excluding people/markers with extremely low call rate...

7390 markers and 918 people in total

0 people excluded because of call rate < 0.1

0 markers excluded because of call rate < 0.1

Passed: 7390 markers and 918 people

Running sex chromosome checks...

0 heterozygous X-linked male genotypes found

0 X-linked markers are likely to be autosomal (odds > 1000 )

0 male are likely to be female (odds > 1000 )

0 female are likely to be male (odds > 1000 )

0 people have intermediate X-chromosome inbreeding ( $0.5 > F > 0.5$ )

If these people/markers are removed, 0 heterozygous male genotypes are left

Passed: 7390 markers and 918 people

no X/Y/mtDNA-errors to fix

RUN 1

7390 markers and 918 people in total

0 (0%) markers excluded as having low (<0.2723312%) minor allele frequency

0 (0%) markers excluded because of low (<95%) call rate

0 (0%) markers excluded because they are out of HWE ( $P < 0$ )

```

0 (0%) people excluded because of low (<95%) call rate
Mean autosomal HET is 0.2562716 (s.e. 0.0151377)
0 people excluded because too high autosomal heterozygosity (FDR <1%)
Mean IBS is 0.784678 (s.e. 0.01510465), as based on 2000 autosomal markers
0 (0%) people excluded because of too high IBS (>=0.95)
In total, 7390 (100%) markers passed all criteria
In total, 918 (100%) people passed all criteria

```

```
> summary(qc2)
```

```
$`Per-SNP fails statistics`
```

	NoCall	NoMAF	NoHWE	Redundant	Xsnpfail
NoCall	0	0	0	0	0
NoMAF	NA	0	0	0	0
NoHWE	NA	NA	0	0	0
Redundant	NA	NA	NA	0	0
Xsnpfail	NA	NA	NA	NA	0

```
$`Per-person fails statistics`
```

	IDnoCall	HetFail	IBSFail	isfemale	ismale	isXXY	otherSexErr
IDnoCall	0	0	0	0	0	0	0
HetFail	NA	0	0	0	0	0	0
IBSFail	NA	NA	0	0	0	0	0
isfemale	NA	NA	NA	0	0	0	0
ismale	NA	NA	NA	NA	0	0	0
isXXY	NA	NA	NA	NA	NA	0	0
otherSexErr	NA	NA	NA	NA	NA	NA	0

**Answer (Ex. 6)** — The list of genetic females who are coded as males is

```
> qc1$isfemale
```

```

[1] "id3374" "id6263" "id6835" "id8410" "id8509" "id8519" "id8542" "id2701"
[9] "id6494" "id3100"

```

**Answer (Ex. 7)** — The list of genetic males who are coded as females is

```
> qc1$ismale
```

```
[1] "id193" "id8475" "id2461" "id5669" "id7245" "id8301"
```

**Answer (Ex. 8)** — The number of 'XXY' people is 0

**Answer (Ex. 9)** — Eight 'sporadic' X-errors are left after removing people with likely sex code errors (seven in the data set after first step of QC)

**Answer (Ex. 10)** — The list of IDs failing IBS checks ('twin' DNAs) is

```
> qc1$ibsfail
```

```
[1] "id3368" "id9668" "id5437" "id956" "id386" "id660" "id2115" "id8370"
```

**Answer (Ex. 11)** — The second step of QC:

```
> data1.gkin <- ibs(data1[,autosomal(data1)],weight="freq")
> data1.dist <- as.dist(0.5-data1.gkin)
> data1.mds <- cmdscale(data1.dist)
> km <- kmeans(data1.mds,centers=2,nstart=1000)
> cl1 <- names(which(km$cluster==1))
> cl2 <- names(which(km$cluster==2))
> if (length(cl1)>length(cl2)) {x<-cl2;cl2<-cl1;cl1<-x}
> cl1
```

```
[1] "id2097" "id2126" "id2878" "id3021" "id3176" "id4554" "id4756" "id7436"
[9] "id7533" "id9396" "id9546" "id4021" "id2171" "id6954" "id2136" "id5056"
[17] "id1751" "id6626" "id2970" "id1300" "id8639" "id1729" "id9398" "id9904"
[25] "id858"
```

```
> data2 <- data1[cl2,]
> qc2 <- check.marker(data2,hweids=(phdata(data2)$dm2==0),fdr=0.2)
```

Excluding people/markers with extremely low call rate...

7390 markers and 893 people in total

0 people excluded because of call rate < 0.1

0 markers excluded because of call rate < 0.1

Passed: 7390 markers and 893 people

Running sex chromosome checks...

0 heterozygous X-linked male genotypes found

0 X-linked markers are likely to be autosomal (odds > 1000 )

0 male are likely to be female (odds > 1000 )

0 female are likely to be male (odds > 1000 )

0 people have intermediate X-chromosome inbreeding ( $0.5 > F > 0.5$ )

If these people/markers are removed, 0 heterozygous male genotypes are left

Passed: 7390 markers and 893 people

no X/Y/mtDNA-errors to fix

RUN 1

7390 markers and 893 people in total

5 (0.067659%) markers excluded as having low (<0.2799552%) minor allele frequency

0 (0%) markers excluded because of low (<95%) call rate

0 (0%) markers excluded because they are out of HWE (FDR <0.2)

0 (0%) people excluded because of low (<95%) call rate

Mean autosomal HET is 0.2565083 (s.e. 0.01505982)

0 people excluded because too high autosomal heterozygosity (FDR <1%)

Mean IBS is 0.7924553 (s.e. 0.01155493), as based on 2000 autosomal markers

0 (0%) people excluded because of too high IBS ( $\geq 0.95$ )

In total, 7385 (99.93234%) markers passed all criteria

In total, 893 (100%) people passed all criteria

```

RUN 2
7385 markers and 893 people in total
0 (0%) markers excluded as having low (<0.2799552%) minor allele frequency
0 (0%) markers excluded because of low (<95%) call rate
0 (0%) markers excluded because they are out of HWE (FDR <0.2)
0 (0%) people excluded because of low (<95%) call rate
Mean autosomal HET is 0.2565083 (s.e. 0.01505982)
0 people excluded because too high autosomal heterozygosity (FDR <1%)
Mean IBS is 0.7886722 (s.e. 0.01162941), as based on 2000 autosomal markers
0 (0%) people excluded because of too high IBS (>=0.95)
In total, 7385 (100%) markers passed all criteria
In total, 893 (100%) people passed all criteria

> summary(qc2)

$`Per-SNP fails statistics`
      NoCall NoMAF NoHWE Redundant Xsnpfail
NoCall      0      0      0          0        0
NoMAF       NA      5      0          0        0
NoHWE       NA     NA      0          0        0
Redundant   NA     NA     NA          0        0
Xsnpfail    NA     NA     NA          NA        0

$`Per-person fails statistics`
      IDnoCall HetFail IBSFail isfemale ismale isXXY otherSexErr
IDnoCall      0      0      0          0      0      0          0
HetFail       NA      0      0          0      0      0          0
IBSFail       NA     NA      0          0      0      0          0
isfemale      NA     NA     NA          0      0      0          0
ismale        NA     NA     NA          NA      0      0          0
isXXY         NA     NA     NA          NA     NA      0          0
otherSexErr    NA     NA     NA          NA     NA     NA          0

> data2 <- data2[qc2$idok,qc2$snpok]
> data2 <- Xfix(data2)

no X/Y/mtDNA-errors to fix

> ####
> #ge03d2.clean <- data2
> #save(ge03d2.clean,file="ge03d2.clean.RData")
> ####

```

**Answer (Ex. 12)** — The list of genetic outliers is

```

> cl1
[1] "id2097" "id2126" "id2878" "id3021" "id3176" "id4554" "id4756" "id7436"
[9] "id7533" "id9396" "id9546" "id4021" "id2171" "id6954" "id2136" "id5056"
[17] "id1751" "id6626" "id2970" "id1300" "id8639" "id1729" "id9398" "id9904"
[25] "id858"

```

**Answer (Ex. 13) — :**

```
> table(phdata(data2)$dm2)
  0  1
472 421
```

**Answer (Ex. 14) — :**

```
> nsnps(data2)
[1] 7385
```

**Answer (Ex. 15) — No:**

```
> descriptives.marker(data2)[2]
$`Cumulative distr. of number of SNPs out of HWE, at different alpha`
      X<=1e-04 X<=0.001 X<=0.01 X<=0.05 all X
No           1      4.000  44.000 258.000  7385
Prop          0      0.001   0.006   0.035    1
```

**Answer (Ex. 16) — :**

```
> qts <- qtscore(dm2,data2,trait="binomial")
> lambda(qts)
$estimate
[1] 1.137922
```

```
$se
[1] 0.001087627
```

```
$iz0
[1] 1.131274
```

```
$iz2
[1] 1
```

**Answer (Ex. 17) — :**

```
> res1 <- qtscore(dm2,data=data2,times=200,quiet=TRUE,trait="binomial")
|
|
|
|=====| 100%
> ds1 <- descriptives.scan(res1)
Summary for top 10 results, sorted by P1df
> ds1
```

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB	chi2.1df
rs7903146	1	1047389	+	A	T	887	0.4732487	0.09237813	26.24463
rs289981	1	1043860	-	T	G	882	1.6084203	0.35859445	20.11833
rs3436694	2	8921418	-	C	G	885	2.0983499	0.47455324	19.55176
rs70099	2	8857747	+	C	A	883	2.3164180	0.52491058	19.47436
rs7064741	1	1044233	-	C	G	885	0.6175678	0.14988526	16.97663
rs2975760	3	10518480	+	A	T	888	1.4799188	0.38810107	14.54073
rs3074653	2	8915495	-	G	C	885	1.6536317	0.44797502	13.62605
rs5243830	1	1371499	+	A	G	888	16.2973905	4.53947050	12.88919
rs5743183	1	648911	+	C	T	882	0.5025524	0.14066512	12.76409
rs9386314	1	756075	-	C	A	889	0.6716160	0.19058053	12.41895
	P1df	Pc1df	effAB	effBB	chi2.2df	P2df			
rs7903146	0.004975124	0.005	0.5218925	0.1037251	26.80404	0.005			
rs289981	0.020000000	0.080	1.3125000	2.3066907	21.00192	0.070			
rs3436694	0.025000000	0.105	1.9610224	Inf	20.49069	0.095			
rs70099	0.030000000	0.115	2.1524086	Inf	20.04047	0.110			
rs7064741	0.125000000	0.355	0.5769088	0.4686275	17.83121	0.265			
rs2975760	0.405000000	0.775	1.1013919	3.3706932	22.84103	0.035			
rs3074653	0.605000000	0.875	1.5340654	4.0687285	14.31287	0.900			
rs5243830	0.765000000	0.925	Inf	Inf	13.73963	0.960			
rs5743183	0.775000000	0.930	0.4646406	0.6684303	13.97357	0.955			
rs9386314	0.820000000	0.950	0.5847590	0.6154026	14.99598	0.795			

There are SNPs which are empirically genome-wide significant in the data. To get the list of 'top' 10 SNPs:

```
> snps1 <- rownames(ds1)
> snps1
[1] "rs7903146" "rs289981" "rs3436694" "rs70099" "rs7064741" "rs2975760"
[7] "rs3074653" "rs5243830" "rs5743183" "rs9386314"
```

**Answer (Ex. 18)** — There is little overlap between SNPs before and after QC:

```
> snps0
[1] "rs2689086" "rs4950080" "rs6386070" "rs7539209" "rs7903146" "rs9350372"
[7] "rs3977642" "rs3880649" "rs6581033" "rs3845221"
> snps1
[1] "rs7903146" "rs289981" "rs3436694" "rs70099" "rs7064741" "rs2975760"
[7] "rs3074653" "rs5243830" "rs5743183" "rs9386314"
```

**Answer (Ex. 19)** — :

```
> data(ge03d2c)
> snps1
[1] "rs7903146" "rs289981" "rs3436694" "rs70099" "rs7064741" "rs2975760"
[7] "rs3074653" "rs5243830" "rs5743183" "rs9386314"
> confdat <- ge03d2c[,snps1]
> rep <- qtscore(dm2,confdat,times=10000,quiet=TRUE)
```

```

|
|
|
|=====| 100%

```

> `descriptives.scan(rep)`

Summary for top 10 results, sorted by P1df

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB
rs2975760	3	10518480	+	A	T	192	0.16759919	0.05487999
rs7903146	1	1047389	+	A	T	191	-0.20235475	0.07195827
rs289981	1	1043860	-	T	G	193	0.13512064	0.05646226
rs7064741	1	1044233	-	C	G	193	-0.13146201	0.05935313
rs3436694	2	8921418	-	C	G	193	0.17717855	0.08512525
rs3074653	2	8915495	-	G	C	190	0.15567151	0.07620495
rs5743183	1	648911	+	C	T	191	-0.09809264	0.08440061
rs9386314	1	756075	-	C	A	193	-0.05607552	0.06194938
rs70099	2	8857747	+	C	A	192	0.04474665	0.10180430
rs5243830	1	1371499	+	A	G	195	0.04527559	0.17760398

	chi2.1df	P1df	Pc1df	effAB	effBB	chi2.2df	P2df
rs2975760	9.32643863	0.0149	0.6015	0.2363080345	0.24878575	10.905443	0.0218
rs7903146	7.90797784	0.0368	0.6889	-0.2327637890	-0.25359712	8.443328	0.0827
rs289981	5.72699574	0.1380	0.8184	0.0166666667	0.19727891	6.586622	0.2154
rs7064741	4.90583841	0.1946	0.8825	-0.2232316677	-0.06719368	8.569669	0.0781
rs3436694	4.33216994	0.2630	0.9288	0.1221340388	0.58641975	5.316940	0.4203
rs3074653	4.17303421	0.2919	0.9326	0.1088501292	0.59722222	5.524265	0.3825
rs5743183	1.35077106	0.9162	0.9976	-0.1142919106	-0.12578616	1.440586	0.9971
rs9386314	0.81935620	0.9811	0.9994	-0.0948499882	0.01960784	1.790760	0.9915
rs70099	0.19319191	1.0000	1.0000	0.0002587992	0.56547619	1.292451	0.9999
rs5243830	0.06498646	1.0000	1.0000	-0.1921052632	0.55789474	1.860430	0.9885

**Answer (Ex. 20)** — Two-stage P-value is

```

> snps1
[1] "rs7903146" "rs289981" "rs3436694" "rs70099" "rs7064741" "rs2975760"
[7] "rs3074653" "rs5243830" "rs5743183" "rs9386314"

> finres <- matrix(NA,10,3)
> colnames(finres) <- c("Stage 1","Replication","Combined")
> rownames(finres) <- snps1
> for (i in 1:10) {
+   finres[i,1] <- res1[which(snpnames(data2)==snps1[i]),"Pc1df"]
+   finres[i,2] <- rep[which(snpnames(confdat)==snps1[i]),"P1df"]
+   finres[i,3] <- finres[i,1]*finres[i,2]
+ }
> finres

```

	Stage 1	Replication	Combined
rs7903146	0.005	0.0368	0.0001840
rs289981	0.080	0.1380	0.0110400
rs3436694	0.105	0.2630	0.0276150
rs70099	0.115	1.0000	0.1150000

```

rs7064741    0.355      0.1946 0.0690830
rs2975760    0.775      0.0149 0.0115475
rs3074653    0.875      0.2919 0.2554125
rs5243830    0.925      1.0000 0.9250000
rs5743183    0.930      0.9162 0.8520660
rs9386314    0.950      0.9811 0.9320450

> for (i in 1:10) {
+   if (finres[i,3] <= 0.05) {
+     print(c("-----",rownames(finres)[i],"-----"))
+
+     print(c(rownames(finres)[i],"stage 1:"))
+     ph <- phdata(data2)$dm2
+     gt <- as.numeric(data2[,rownames(finres)[i]])
+     print(summary(glm(ph~gt,family=binomial))$coef)
+
+     print(c(rownames(finres)[i],"stage 2:"))
+     ph <- phdata(confdat)$dm2
+     gt <- as.numeric(confdat[,rownames(finres)[i]])
+     print(summary(glm(ph~gt,family=binomial))$coef)
+
+     print(c(rownames(finres)[i],"Joint:"))
+     ph <- c(phdata(data2)$dm2,phdata(confdat)$dm2)
+     gt <- c(as.numeric(data2[,rownames(finres)[i]]),as.numeric(confdat[,rownames(finres)[i]]))
+     print(summary(glm(ph~gt,family=binomial))$coef)
+   }
+ }

[1] "-----" "rs7903146" "-----"
[1] "rs7903146" "stage 1:"
      Estimate Std. Error  z value    Pr(>|z|)
(Intercept)  0.08024472 0.07682467  1.044518 2.962460e-01
gt          -0.74685964 0.14906771 -5.010204 5.437238e-07
[1] "rs7903146" "stage 2:"
      Estimate Std. Error  z value    Pr(>|z|)
(Intercept)  0.001895645  0.1686559  0.01123972 0.991032189
gt          -0.902521348  0.3296467 -2.73784449 0.006184331
[1] "rs7903146" "Joint:"
      Estimate Std. Error  z value    Pr(>|z|)
(Intercept)  0.06652706 0.06990581  0.9516671 3.412659e-01
gt          -0.77557677 0.13580221 -5.7110762 1.122640e-08
[1] "-----" "rs289981" "-----"
[1] "rs289981" "stage 1:"
      Estimate Std. Error  z value    Pr(>|z|)
(Intercept) -0.7895683  0.1686946 -4.680461 2.862312e-06
gt           0.4836336  0.1088004  4.445145 8.783281e-06
[1] "rs289981" "stage 2:"
      Estimate Std. Error  z value    Pr(>|z|)
(Intercept) -1.0644645  0.383275 -2.777287 0.005481483
gt           0.5699411  0.241041  2.364499 0.018054487
[1] "rs289981" "Joint:"

```



```

      Estimate Std. Error   z value    Pr(>|z|)
(Intercept) -0.8342403  0.1542809 -5.407281 6.398868e-08
gt          0.4963232  0.0990676  5.009944 5.444577e-07
[1] "-----" "rs3436694" "-----"
[1] "rs3436694" "stage 1:"
      Estimate Std. Error   z value    Pr(>|z|)
(Intercept) -0.2592162  0.07519988 -3.447029 5.667876e-04
gt          0.7385083  0.16987721  4.347307 1.378194e-05
[1] "rs3436694" "stage 2:"
      Estimate Std. Error   z value    Pr(>|z|)
(Intercept) -0.3683936  0.1589513 -2.317651 0.02046831
gt          0.7304292  0.3606365  2.025389 0.04282744
[1] "rs3436694" "Joint:"
      Estimate Std. Error   z value    Pr(>|z|)
(Intercept) -0.2790299  0.06795526 -4.106083 4.024252e-05
gt          0.7391791  0.15373558  4.808120 1.523565e-06
[1] "-----" "rs2975760" "-----"
[1] "rs2975760" "stage 1:"
      Estimate Std. Error   z value    Pr(>|z|)
(Intercept) -0.3171429  0.08729818 -3.632870 0.0002802862
gt          0.3944701  0.10419297  3.785957 0.0001531181
[1] "rs2975760" "stage 2:"
      Estimate Std. Error   z value    Pr(>|z|)
(Intercept) -0.5969939  0.1928321 -3.095926 0.001961992
gt          0.6942268  0.2323045  2.988435 0.002804103
[1] "rs2975760" "Joint:"
      Estimate Std. Error   z value    Pr(>|z|)
(Intercept) -0.3659711  0.07942275 -4.607888 4.067798e-06
gt          0.4463331  0.09495765  4.700338 2.597310e-06

> replicatedsnps <- rownames(finres)[finres[, "Stage 1"] <= 0.05 &
+                               finres[, "Replication"] <= 0.05 & finres[, "Combined"] <= 0.05]
> replicatedsnps
[1] "rs7903146"

> sigsnps <- rownames(finres)[finres[, "Combined"] <= 0.05]
> sigsnps
[1] "rs7903146" "rs289981" "rs3436694" "rs2975760"

```

At the first glance, 1 SNPs may be claimed as replicated because both first stage and replication  $P$ -values are  $\leq 0.05$  and effects are consistent, and additionally 3 may be claimed as 'significant' because joint  $p$ -values are  $\leq 0.05$  and the effects are consistent. Generally, a more thorough simulation experiment should be performed.

**Answer (Ex. 21)** — SNP "rs7903146" had empirical  $p$ -value  $\leq 0.05$  at both stages, and very strong joint significance. It can be claimed as replicated.

You can check if any of the SNPs you have identified as significant or replicated are the ones which were simulated to be associated with `dm2` by using the command `show.ncbi(c("snpname1", "snpname2", "snpname3"))` where `snpnameX`

stands for the name of your identified SNP. The "true" SNPs can be found on NCBI and some are located in known T2D genes (just because we used these names to name the "significant" ones).

## Chapter 6

# GWA analysis in presence of stratification: theory

In genetic association studies, we look for association between a genetic polymorphism and the value of a trait of interest. The best scenario – the one we always hope for – is that the observed association results from causation, that is the polymorphism studied is functionally involved in the control of the trait. However, association has no direction, and making causal inference in epidemiology in general and in genetic epidemiology in particular is usually not possible based on statistical analysis only.

In fact, most associations observed in genetic studies are due to a confounder – an (unobserved) factor which is associated with both the genetic polymorphism and the trait analysed. Presence of such factor leads to induced, “secondary” correlation between the trait and the polymorphism; if we would have controlled for that factor in the association model, the relation between the polymorphism and the trait would have gone.

There are two major types of confounders leading to induced correlation in genetic association studies. One type is “good” confounding of association by the real, unobserved functional variant, which is, as a rule, not present on the SNP array, but is in linkage disequilibrium (LD) with typed SNP. Under this scenario, the functional variant is associated with the trait because of causative relation; at the same time it is associated with a typed polymorphism located nearby because of LD. This confounding induces secondary correlation between the typed polymorphism and the trait, making localisation of the true functional polymorphism (LD mapping) possible.

Other major type of confounding observed in genetic association studies is confounding by population (sub)structure. Let us consider a study in which subjects come from two distantly related populations, say Chinese and European. Due to genetic drift, these two populations will have very different frequencies at many loci throughout the genome. At the same time, these two populations are different phenotypically (prevalence of different disease, mean value of quantitative trait) due to accumulated genetic and cultural differences. Therefore any of these traits will show association with multiple genomic loci. While some of these associations may be genuine genetic associations in a sense that either the polymorphisms themselves, or the polymorphisms close by are causally

involved, most of these associations will be genetically false positives – noise associations generated by strong genetic and phenotypic divergence between the two populations.

The scenario described above is extreme and indeed it is hard to imagine a genetic association study in which two very distinct populations are so blantly mixed and analysed not taking this mixture into account. However, a more subtle scenario where several slightly genetically different populations are mixed in the same study is frequently the case and a matter of concern in GWA studies.

In this chapter, we will define what is genetic structure, and how it can be quantified (section 6.1); what are the effects of genetic structure on the standard association tests (section 6.2) and specific association tests which take possible genetic structure into account (section 6.3).

## 6.1 Genetic structure of populations

A major unit of genetic structure is a genetic population. Different definitions of genetic population are available, for example Wikipedia defines population (biol.) as "the collection of inter-breeding organisms of a particular species". The genetics of populations is "the study of the allele frequency distribution and change under the influence of ... evolutionary processes". In the framework of population genetics, the main characteristics of interest of a group of individuals are their genotypes, frequencies of alleles in this group, and the dynamics of these distributions in time. While the units of interest of population genetics are alleles, the units of evolutionary processes are acting upon are organisms. Therefore a definition of a genetic population should be based on the chance that different alleles, present in the individuals in question can mix together; if such chance is zero, we may consider such groups as different populations, each described by its own genotypic and allelic frequencies and their dynamic. Based on these considerations, a genetic population may be defined in the following way:

*Two individuals,  $I_1$  and  $I_2$ , belong to the same population if (a) the probability that they would have an offspring in common is greater than zero and (b) this probability is much higher than the probability of  $I_1$  and  $I_2$  having an offspring in common with some individual  $I_3$ , which is said to belong to other genetic population.*

Here, to have an offspring in common does not imply having a direct offspring, but rather a common descendant in a number of generations.

However, in gene discovery in general and GWA studies in particular we are usually not interested in future dynamics of alleles and genotypes distributions. What is the matter of concern in genetic association studies is potential common ancestry – that is that individuals may share common ancestors and thus share in common the alleles, which are exact copies of the same ancestral allele. Such alleles are called "identical-by-descent", or IBD for short. If the chance of IBD is high, this reflects high degree of genetic relationship. As a rule, relatives share many features, both environmental and genetic, which may lead to confounding.

Genetic relationship between a pair of individuals is quantified using the "coefficient of kinship", which measures that chance that gametes, sampled at random from these individuals, are IBD.

Thus for the purposes of gene-discovery we can define genetic population use retrospective terms and based on the concept of IBD:

*Two individuals,  $I_1$  and  $I_2$ , belong to the same genetic population if (a) their genetic relationship, measured with the coefficient of kinship, is greater than zero and (b) their kinship is much higher than kinship between them and some individual  $I_3$ , which is said to belong to other genetic population.*

One can see that this definition is quantitative and rather flexible (if not to say arbitrary): what we call a "population" depends on the choice of the threshold for the "much-higher" probability. Actually, what you define as "the same" genetic population depends in large part on the scope aims of your study. In human genetics literature you may find references to a particular genetically isolated population, population of some country (e.g. "German population", "population of United Kingdom"), European, Caucasoid or even general human population. Defining a population is about deciding on some probability threshold.

In genetic association studies, it is frequently assumed that study participants are "unrelated" and "come from the same genetic population". Here, "unrelated" means, that while study participants come from the same population (so, there is non-zero kinship between them!), this kinship is so low that it has very little effect on the statistical testing procedures used to study association between genes and phenotypes.

In the following sections we will consider the effects of population structure on the distribution of genotypes in a study population. We will start with assumption of zero kinship between study participants, which would allow us to formulate Hary-Weinberg principle (section 6.1.1). In effect, there is no such thing as zero kinship between any two organisms, however, when kinship is very low, the effects of kinship on genotypic distribution are minimal, as we will see in section 6.1.2. The effects of substructure – that is when study sample consist of several genetic populations – onto genotypic distribution will be considered in section 6.1.3. Finally, we will generalize the obtained results for the case of arbitrary structures and will see what are the effects of kinship onto joint distribution of genotypes and phenotypes in section ??.

### 6.1.1 Hardy-Weinberg equilibrium

To describe genetic structure of populations we will use rather simplistic model approximating genetic processes in natural populations. Firstly, we will assume that the population under consideration has infinitely large size, which implies that we can work in terms of probabilities, and no random process take place. Secondly, we accept non-overlapping

$$\text{generation} \Rightarrow \text{gametic pool} \Rightarrow \text{generation}$$

model. This model assumes that a set of individuals contributes gametes to genetic pool, and dies out. The gametes are sampled randomly from this pool in pairs to form individuals of the second generation. The selection acts on individuals, while mutation occurs when the gametic pool is formed. The key point of this model is the abstract of gametic pool: if you use that, you do not need to consider all pair-wise mating between male and female individuals; you rather

consider some abstract infinitely large pool, where gametes are contributed to with the frequency proportional to that in previous generation. Interestingly, this rather artificial construct has a great potential to describe the phenomena we indeed observe in nature.

In this section, we will derive Hardy-Weinberg law (this analog of the Mendel's law for populations). The question to be answered is, if some alleles at some locus segregate according to Mendel's laws and aggregate totally at random, what would be genotypic distribution in a population?

Let us consider two alleles, wild type normal allele ( $N$ ) and a mutant ( $D$ ), segregating at some locus in the population and apply the "generation  $\Rightarrow$  gametic pool  $\Rightarrow$  generation" model. Let us denote the frequency of the  $D$  allele in the gametic pool as  $q$ , and the frequency of the other allele,  $N$ , as  $p = 1 - q$ . Gametes containing alleles  $N$  and  $D$  are sampled at random to form diploid individuals of the next generation. The probability to sample a " $N$ " gamete is  $p$ , and the probability that the second sampled gamete is also " $N$ " is also  $p$ . According to the rule, which states that joint probability of two independent events is a product of their probabilities, the probability to sample " $N$ " and " $N$ " is  $p \cdot p = p^2$ . In the same manner, the probability to sample " $D$ " and then " $D$ " is  $q \cdot q = q^2$ . The probability to sample first the mutant and then normal allele is  $q \cdot p$ , the same is the probability to sample " $D$ " first and " $N$ " second. In most situations, we do not (and can not) distinguish heterozygous genotypes  $DN$  and  $ND$  and refer to both of them as " $ND$ ". In this notation, frequency of  $ND$  will be  $q \cdot p + p \cdot q = 2 \cdot p \cdot q$ . Thus, we have computed the genotypic distribution for a population formed from a gametic pool in which the frequency of  $D$  allele was  $q$ .

To obtain the next generation, the next gametic pool is generated. The frequency of  $D$  in the next gametic pool is  $q^2 + \frac{1}{2} \cdot 2 \cdot p \cdot q$ . Here,  $q^2$  is the probability that a gamete-contributing individual has genotype  $DD$ ;  $2 \cdot p \cdot q$  is the probability that a gamete-contributing individual is  $ND$ , and  $\frac{1}{2}$  is the probability that  $ND$  individual contributes  $D$  allele (only half of the gametes contributed by individuals with  $ND$  genotype are  $D$ ); see Figure 6.1. Thus the frequency of  $D$  in the gametic pool is  $q^2 + \frac{1}{2} \cdot 2 \cdot p \cdot q = q \cdot (q + p) = q$  — exactly the same as it was in previous gametic pool.

Thus, if assumptions of random segregation and aggregation hold, the expected frequency of  $NN$ ,  $ND$  and  $DD$  genotypes are stable over generations and can be related to the allelic frequencies using the following relation

$$\begin{aligned} P(NN) &= (1 - q) \cdot (1 - q) &= p^2, \\ P(ND) &= q \cdot (1 - q) + (1 - q) \cdot q &= 2 \cdot p \cdot q, \\ P(DD) &= q \cdot q &= q^2 \end{aligned} \tag{6.1}$$

which is known as Hardy-Weinberg equilibrium (HWE) point.

There are many reasons, in which random segregation and aggregation, and, consequently, Hardy-Weinberg equilibrium, are violated. It is very important to realize that, especially if the study participants are believed to come from the same genetic population, most of the times when deviation from HWE is detected, this deviation is due to technical reasons, i.e. genotyping error. Therefore testing for HWE is a part of the genotypic quality control procedure in most studies. Only when the possibility of technical errors is eliminated, other possible explanations may be considered. In a case when deviation from HWE can

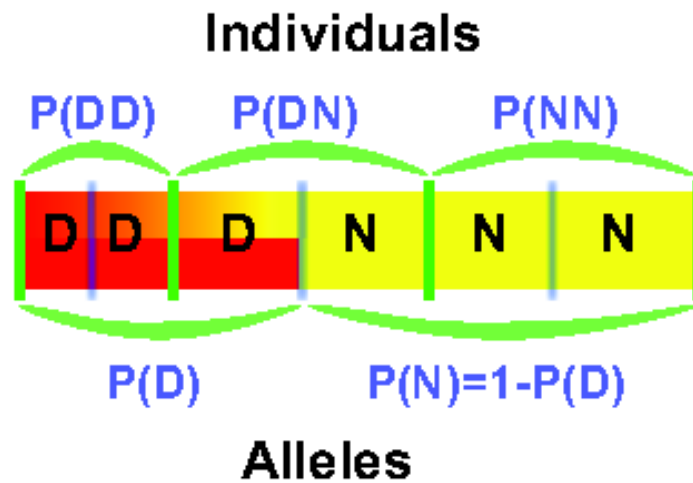


Figure 6.1: Genotypic and allelic frequency distribution in a population;  $q = P(D) = P(DD) + \frac{1}{2} \cdot P(DN)$ .

not be explained by technical reasons, the most frequent explanation would be that the sample tested is composed of representatives of different genetic populations, or more subtle genetic structure. However, unless study participants represent a mixture of very distinct genetic populations – the chances of which coming unnoticed are low – the effects of genetic structure on HWE are difficult to detect, at least for any single marker, as you will see in the next sections.

### 6.1.2 Inbreeding

Inbreeding is preferential breeding between (close) relatives. An extreme example of inbreeding is a selfing, a breeding system, observed in some plants. The inbreeding is not uncommon in animal and human populations. Here, the main reason for inbreeding are usually geographical (e.g. mice live in very small interbred colonies – dems – which are usually established by few mice and are quite separated from other dems) or cultural (e.g. noble families of Europe).

Clearly, such preferential breeding between relatives violates the assumption of random aggregation, underling Hardy-Weinberg principle. Relatives are likely to share the same alleles, inherited from common ancestors. Therefore their progeny has an increased chance of being *autozygous* – that is to inherit a copy of exactly the same ancestral allele from both parents. An autozygous genotype is always homozygous, therefore inbreeding should increase the frequency of homozygous, and decrease the frequency of heterozygous, genotypes.

Inbreeding is quantified by the *coefficient of inbreeding*, which is defined as the probability of autozygosity. This coefficient may characterize an individual, or a population in general, in which case this is expectation that a random individual from the population is autozygous at a random locus. The coefficient of inbreeding is closely related to the coefficient of kinship, defined earlier for a pair of individuals as the probability that two alleles sampled at random from these individuals, are IBD. It is easy to see that the coefficient of inbreeding for





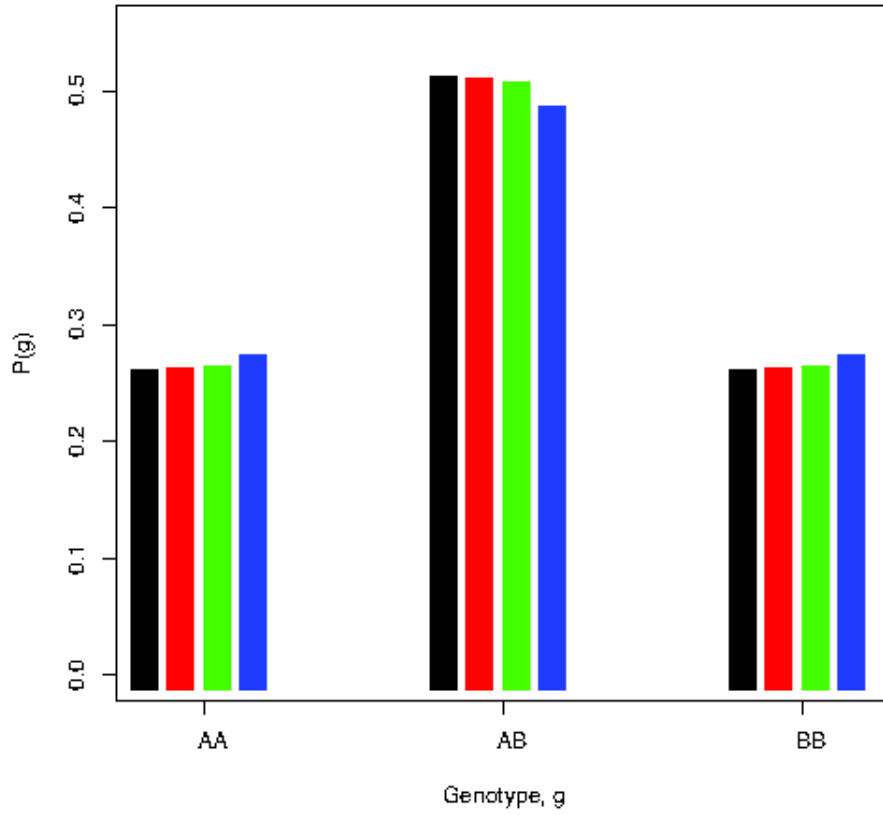


Figure 6.3: Genotypic probability distribution for a locus with 50% frequency of the  $B$  allele; black bar, no inbreeding; red,  $F = 0.001$ ; green,  $F = 0.01$ ; blue,  $F = 0.05$

it is not inbred ( $1 - F$ ), and the second term corresponds to probability that a person is  $AA$  given it is inbred ( $p$ ), multiplied by the probability that the person is inbred ( $F$ ). This computations can be easily done for all genotypic classes leading to the expression for HWE under inbreeding.

$$\begin{aligned}
 P(AA) &= (1 - F) \cdot p^2 + F \cdot p &= p^2 + p \cdot q \cdot F \\
 P(AB) &= (1 - F) \cdot 2 \cdot p \cdot q + F \cdot 0 &= 2 \cdot p \cdot q \cdot (1 - F) \\
 P(BB) &= (1 - F) \cdot q^2 + F \cdot q &= q^2 + p \cdot q \cdot F
 \end{aligned} \tag{6.2}$$

How much is inbreeding expected to modify genotypic distribution in human populations? The levels of inbreeding observed in human genetically isolated populations typically vary between 0.001 (low inbreeding) to 0.05 (relatively high), see PARDO *et al.* (2005); RUDAN *et al.* (2003). The genotypic distribution for  $q = 0.5$  and different values of the inbreeding coefficient is shown at the figure 6.3.

What is the power to detect deviation from HWE due to inbreeding? For that, we need to estimate the expectation of the  $\chi^2$  statistics (the non-centrality parameter, NCP) used to test for HWE. The test for HWE is performed using

standard formula

$$T^2 = \sum_i \frac{(O_i - E_i)^2}{E_i} \quad (6.3)$$

where summation is performed over all classes (genotypes);  $O_i$  is the count observed in  $i$ -th class, and  $E_i$  is the count expected under the null hypothesis (HWE). Under the null hypothesis, this test statistic is distributed as  $\chi^2$  with number of degrees of freedom equal to the number of genotypes minus the number of alleles.

Thus the expectation of this test statistic for some  $q$ ,  $F$ , and  $N$  (sample size) is

$$\begin{aligned} E[T^2] &= \frac{(N(q^2 + pqF) - Nq^2)^2}{Nq^2} + \frac{(N2pq(1-F) - N2pq)^2}{N2pq} + \frac{(N(p^2 + pqF) - Np^2)^2}{Np^2} \\ &= \frac{(NpqF)^2}{Nq^2} + \frac{(-2NpqF)^2}{N2pq} + \frac{(NpqF)^2}{Np^2} \\ &= Np^2F^2 + 2NpqF^2 + Nq^2F^2 \\ &= NF^2(p^2 + 2pq + q^2) \\ &= N \cdot F^2 \end{aligned} \quad (6.4)$$

Interestingly, the non-centrality parameter does not depend on the allelic frequency. Given the non-centrality parameter, it is easy to compute the power to detect deviation from HWE for any given  $F$ . For example, to achieve the power of  $> 0.8$  at  $\alpha = 0.05$ , for a test with one degree of freedom the non-centrality parameter should be  $> 7.85$ . Thus, if  $F = 0.05$ , to have 80% power,  $N \cdot F^2 > 7.85$ , that is the required sample size should be  $N > \frac{7.85}{F^2} = \frac{7.85}{0.0025} = 3140$  people.

Thus, even in populations with strong inbreeding, rather large sample sizes are required to detect the effects of inbreeding on HWE at a particular locus, even at relatively weak significance level of 5%.

While the chance that deviation from HWE due to inbreeding will be statistically significant is relatively small, inbreeding may have clear effects on the results of HWE testing in GWA study. Basically, if testing is performed at a threshold corresponding to nominal significance  $\alpha$ , a proportion of markers which show significant deviation will be larger than  $\alpha$ . Clearly, how large this proportion will be depends on the inbreeding and on size of the study – expectation of  $T^2$  is a function of both  $N$  and  $F$ . A proportion of markers showing significant deviation from HWE at different values of inbreeding, sample size, and nominal significance threshold, is shown in table 6.1. While deviation of this proportion from nominal one is minimal at large  $\alpha$ 's and small sample sizes and coefficients of inbreeding, it may be 10-fold and even 100-fold higher than the nominal level at reasonable values of  $N$  and  $F$  for smaller thresholds.

### 6.1.3 Mixture of genetic populations: Wahlund's effect

Consider the following artificial example. Imagine that recruitment of study participants occurs at a hospital, which serves two equally size villages ( $V_1$  and  $V_2$ ); however, the villages are very distinct because of cultural reasons, and most marriages occur within a village. Thus these two villages represent two genetically distinct populations. Let us consider a locus with two alleles,  $A$  and

Table 6.1: Expected proportion of markers deviating from HWE in a sample of  $N$  people coming from a population with average inbreeding  $F$ . Proportion of markers is shown for particular test statistic threshold, corresponding to nominal significance  $\alpha$ .

$N$	$F$	$\alpha$		
		0.05	$10^{-4}$	$5 \cdot 10^{-8}$
1,000	0.001	0.0501	$1.008 \cdot 10^{-4}$	$5.077 \cdot 10^{-8}$
	0.005	0.0529	$1.205 \cdot 10^{-4}$	$7.025 \cdot 10^{-8}$
	0.010	0.0615	$1.885 \cdot 10^{-4}$	$14.503 \cdot 10^{-8}$
10,000	0.001	0.0511	$1.081 \cdot 10^{-4}$	$5.784 \cdot 10^{-8}$
	0.005	0.0790	$3.544 \cdot 10^{-4}$	$36.991 \cdot 10^{-8}$
	0.010	0.1701	$19.231 \cdot 10^{-4}$	$426.745 \cdot 10^{-8}$

Table 6.2: Genotypic proportions in a mixed population

Village	%Sample	$p(A)$	$P(AA)$	$P(AB)$	$P(BB)$
$V_1$	50	0.9	0.81	0.18	0.01
$V_2$	50	0.2	0.04	0.32	0.64
Observed					
Pooled	100	0.55	0.425	0.25	0.325
Expected					
			0.30	0.50	0.20
Difference					
			0.125	-0.250	0.125

$B$ . The frequency of  $A$  is 0.9 in  $V_1$  and it is 0.2 in  $V_2$ . In each population, marriages occur at random, and HWE holds for the locus. What genotypic distribution is expected in a sample ascertained in the hospital, which represents a 1 : 1 mixture of the two populations?

The expected genotypic proportions are presented in table 6.2. First, assuming that HWE holds for each of the populations, we can compute genotypic proportions within these (rows 1 and 2 of table 6.2). If our sample represents a 1 : 1 mixture of these populations, then the frequency of some genotype is also 1 : 1 mixture of the respective frequencies. For example, frequency of  $AA$  genotype would be  $\frac{0.81}{2} + \frac{0.04}{2} = 0.425$ , and so on. The frequency of the  $A$  allele in pooled sample will be  $0.425 + \frac{0.25}{2} = 0.55$ . Based on this frequency we would expect genotypic frequency distribution of 0.3, 0.5 and 0.2, for  $AA$ ,  $AB$ , and  $BB$ , respectively. As you can see the observed distribution has much higher frequencies of homozygous genotypes – excess of homozygotes.

It is notable, that the differences between the observed homozygotes frequencies and these expected under HWE are both 0.125, and, consequently, the observed heterozygosity is less than that expected by  $0.125 \cdot 2 = 0.25$ .

The phenomenon of deviation from HWE due to the fact that considered population consist of two sub-populations, is known as "Wahlund's effect", after the scientist who has first considered and quantified genotypic distribution under such model WAHLUND (1928).

Such marked differences between observed and expected under HWE are very easily detected; for the above example, a sample of  $\approx 35$  people is enough

Table 6.3: Genotypic proportions of *PPARG*GenABEL-package *Pro12Ala* genotype in a mixed population

Ethnicity	%Sample	$p(Pro)$	$P(Pro/Pro)$	$P(Pro/Ala)$	$P(Ala/Ala)$
Caucasian	50	0.85	0.7225	0.2550	0.0225
Afro-American	50	0.99	0.9801	0.0198	0.0001
Observed					
Pooled	100	0.92	0.8513	0.1374	0.0113
Expected					
			0.8464	0.1472	0.0064
Difference					
			0.0049	-0.0098	0.0049

to reject the hypothesis of HWE (power > 80% at  $\alpha = 0.05$ ).

However, the differences we can see in real life are not so marked. For example, the common Pro allele at position 12 of the peroxisome proliferator-activated receptor gamma is associated with increased risk for type 2 diabetes. The frequency of the Pro allele is about 85% in European populations and Caucasian-Americans, about 97% in Japan and 99% in African-American (see table 1 from RUIZ-NARVÁEZ (2005)). Table 6.3 shows hypothetical observed and expected genotypic proportions in a sample composed of 50% Caucasians and 50% African-American.

You can see that observed distribution and the one expected under HWE are very similar; only a sample as large as 1,800 people would allow detection of the deviation from HWE (power > 80% at  $\alpha = 0.05$ ). The situation is similar for most genes observed in real life – while the frequencies may be (or may be not) very different for populations, which diverged long time ago, for relatively close populations expected frequency differences are small and large sample sizes are required to detect deviation from HWE due to Wahlund’s effect at a particular fixed locus.

Let us summarize, what genotypic proportions are expected in a sample, which is a mixture of two populations. Let each population is in HWE, and the frequency of the  $B$  allele is  $q_1$  in population one and  $q_2$  in population two. Let the proportion of individuals coming from population one is  $m$  in the mixed population, and consequently the proportion of individuals from population two is  $(1 - m)$ . The allelic frequencies, and genotypic distributions in the original and mixed populations are presented in tale 6.4.

The frequency of the  $B$  allele in the mixed population is just the weighted average of the allelic frequencies in the two populations,  $\bar{q} = m \cdot q_1 + (1 - m) \cdot q_2$ . Let us denote the frequency of the  $A$  allele as  $\bar{p} = 1 - \bar{q}$ . It can be demonstrated that the genotypic frequency distribution in the mixed sample is the function of the frequency of allele  $B$  in the sample,  $\bar{q}$ , and "disequilibrium" parameter  $D$ :

$$\begin{aligned}
 P(AA) &= \bar{p}^2 + \bar{p} \cdot \bar{q} \cdot F_{st} \\
 P(AB) &= 2 \cdot \bar{p} \cdot \bar{q} \cdot (1 - F_{st}) \\
 P(BB) &= \bar{q}^2 + \bar{p} \cdot \bar{q} \cdot F_{st}
 \end{aligned} \tag{6.5}$$

where

$$F_{st} = \frac{m \cdot (1 - m) \cdot (q_1 - q_2)^2}{\bar{p} \cdot \bar{q}} \tag{6.6}$$

Table 6.4: Expected genotypic proportions in a mixed population;  $F_{st}$  is defined by equation 6.6

Population	Prop.	$p(B)$	$P(AA)$	$P(AB)$	$P(BB)$
$P_1$	$m$	$q_1$	$p_1^2$	$2p_1q_1$	$q_1^2$
$P_2$	$(1 - m)$	$q_2$	$p_2^2$	$2p_2q_2$	$q_2^2$
Observed					
Pooled	1.0	$\bar{q} = mq_1$ $+(1 - m)q_2$	$mp_1^2$ $+(1 - m)p_2^2$	$2mp_1q_1$ $+2(1 - m)p_2q_2$	$mq_1^2$ $+(1 - m)q_2^2$
Expected					
			$\bar{p}^2$	$2\bar{p}\bar{q}$	$\bar{q}^2$
Difference					
			$\bar{p}\bar{q}F_{st}$	$-2\bar{p}\bar{q}F_{st}$	$\bar{p}\bar{q}F_{st}$

You can see that equation 6.5, expressing the genotypic frequencies distribution under Wahlund's effect, is remarkably similar (actually, is specifically re-written in a form similar) to the equation 6.2, expressing the genotypic proportions under the effects of inbreeding. Again, the reason is that  $F_{st}$  (as well as  $F$  of equation 6.2) is easily estimated from the data as the ratio between the observed and expected variances of the genotypic distributions. Then the expected non-centrality parameter for the test of HWE is simply  $N \cdot F_{st}^2$ , where  $N$  is the sample size. Therefore our results concerning the proportion of tests expected to pass a particular significance threshold when genome-wide data are analyzed (table 6.1) hold, with replacement of  $F$  with  $F_{st}$ .

We can compute that the values of  $F_{st}$ , corresponding to the population mixtures presented in tables 6.2 and 6.3 are 0.49 and 0.067, respectively, which gives us a shortcut to estimate the sample size required to detect deviation from HWE due to Wahlund's effect (at  $\alpha = 0.05$  and power 80%):  $N > 7.85/0.49^2 \approx 32$  and  $N > 7.85/0.067^2 \approx 1771$ .

A typical value of  $F_{st}$  for European populations is about 0.002 (up to 0.023 NELIS *et al.* (2009)); very large sample sizes are required to detect deviation from HWE at any given locus at such small  $F_{st}$ 's. However, the effects onto the proportion of markers failing to pass HWE test in GWA may be visibly inflated (table 6.1).

## 6.2 Effects of population structure on standard tests for association

### 6.2.1 Standard tests for genetic association

Standard tests for association between genes and a binary trait are the test for allele frequency difference between cases and controls, and the Armitage's trend test for proportions (that the proportion of cases changes across genotypic groups). For quantitative traits, one of the standard tests is the score test for association, which is closely related – even equivalent – to the Armitage's trend test.

We will start with presenting the study data as 2x3 table, where the rows correspond to the case/control status and columns correspond to genotypic groups, and the cells contain counts of events (table 6.5). For example,  $r_0$  is the number

Table 6.5: Counts of cases and controls with different genotypes

Status	Genotype			Total
	$AA$	$AB$	$BB$	
Case	$r_0$	$r_1$	$r_2$	$R$
Control	$s_0$	$s_1$	$s_2$	$S$
Total	$n_0$	$n_1$	$n_2$	$N$

Table 6.6: Counts of alleles in cases and controls

Status	$A$	$B$	Total
Case	$2 \cdot r_0 + r_1$	$r_1 + 2 \cdot r_2$	$2 \cdot R$
Control	$2 \cdot s_0 + s_1$	$s_1 + 2 \cdot s_2$	$2 \cdot S$
Total	$2 \cdot n_0 + n_1$	$n_1 + 2 \cdot n_2$	$2 \cdot N$

of cases with genotype  $AA$ ,  $s_0$  is the number of controls with genotype  $AA$  and so on.

This table can be re-arranged in a 2x2 allelic table, presented in table 6.6. Each cell of this table contains the counts of alleles present in cases and controls, e.g. total number of  $A$  alleles in cases is  $2 \cdot r_0$  (twice the number of cases who are homozygous for the  $A$  allele) plus the number of  $A$  alleles present in heterozygous cases ( $r_1$ ).

Based on these tables, we can test if the allelic frequency is different between the cases and controls, using standard  $\chi^2$  test, formulated as

$$T^2 = \sum_i \frac{(O_i - E_i)^2}{E_i} \quad (6.7)$$

where summation is performed over all cells (defined by combination of genotype/allele and phenotype);  $O_i$  is the count observed in  $i$ -th class, and  $E_i$  is the count expected under the null hypothesis (equal frequencies in cases and controls). Under the null hypothesis, this test statistic is distributed as  $\chi^2$  with number of degrees of freedom equal to the number of independent classes.

The null hypothesis assumes that the frequency of the  $A$  allele is the same in both cases and controls, and is equal to the frequency for  $A$  in the total sample:  $\bar{p} = \frac{2 \cdot n_0 + n_1}{2 \cdot N}$ . Thus the expected count of  $A$  in cases is  $2 \cdot R \cdot \bar{p}$ , and the expected count of  $B$  alleles is  $2 \cdot R \cdot (1 - \bar{p})$ . Similarly, for cases, the expected count of  $A$  is  $2 \cdot S \cdot \bar{p}$ , and the expected count of  $B$  is  $2 \cdot S \cdot (1 - \bar{p})$ .

Now, for the table 6.6 we can re-write the allelic test as

$$T_A^2 = \frac{((2r_0 + r_1) - 2R\bar{p})^2}{2R\bar{p}} + \frac{((r_1 + 2r_2) - 2R(1 - \bar{p}))^2}{2R(1 - \bar{p})} + \frac{((2s_0 + s_1) - 2S\bar{p})^2}{2S\bar{p}} + \frac{((s_1 + 2s_2) - 2S(1 - \bar{p}))^2}{2S(1 - \bar{p})}$$

With some algebra, it can be demonstrated that this expression simplifies to

$$T_a^2 = \frac{2N\{2N(r_1 + 2r_2) - 2R(n_1 + 2n_2)\}^2}{(2R)2(N - R)\{2N(n_1 + 2n_2) - (n_1 + 2n_2)^2\}} \quad (6.8)$$

Under the null hypothesis that the frequency of alleles is the same in cases and controls (and, as you will see later, that HWE holds in total sample) the test statistic is distributed as  $\chi^2$  with one degree of freedom.

An alternative, Armitage's trend test for proportions, can be used to test the null hypothesis. This test is performed using the 2x3 genotypic table (6.5). The null hypothesis assumes that the frequency of cases is the same in all genotypic groups; alternative is that the frequency is not the same, but are, however, not totally arbitrary. As it follows from the name of the test, a trend in proportions is assumed, that is the frequency of cases among people with heterozygous genotypes  $AB$  should be exactly between the frequencies of cases in two homozygous classes. This hypothesis may be formalized using parameters  $\beta_0$ , the expected frequency of cases in the  $AA$  group, and  $\beta_1$ , the increase in frequency of cases in the  $AB$  group. The expected frequency of cases in the  $AB$  group is then  $(\beta_0 + \beta_1)$ ; the frequency of cases in the  $BB$  group is assumed to be  $(\beta_0 + 2 \cdot \beta_1)$ . Parameters  $p_0$  and  $p_1$  can be estimated using the maximum likelihood;  $\hat{\beta}_1 = \frac{(r_1 + 2r_2) - \bar{p}R}{(n_1 + 4n_2) - \bar{p}N}$ , where  $\bar{p} = \frac{2n_2 + n_1}{2N}$  is the frequency of the  $B$  allele in the total sample. Then,  $\beta_0$  is estimated as  $\hat{\beta}_0 = \frac{R}{N} - \hat{\beta}_1 \bar{p}$ . It can be shown that the chi-square test 6.7 based on these expectations takes the form

$$T_t^2 = \frac{N\{N(r_1 + 2r_2) - R(n_1 + 2n_2)\}^2}{R(N - R)\{N(n_1 + 4n_2) - (n_1 + 2n_2)^2\}} \quad (6.9)$$

It can be shown that  $T_t^2 = T_a^2$  if  $n_1 = 2\sqrt{n_0 \cdot n_2}$ , which is equivalent to the condition that HWE holds exactly, in which case the counts of heterozygotes should be  $2Npq$ , with  $p = \sqrt{n_0/N}$  and  $q = \sqrt{n_2/N}$ . When HWE holds for the total sample, the two tests are (at least asymptotically) equivalent. Thus the test  $T_a^2$  is the test for association in presence of HWE; when HWE does not hold, even in absence of association, the values of  $T_a^2$  are greater than the values of  $T_t^2$  test, possibly leading to false positive conclusions about association in presence of deviations from HWE. Therefore the trend test  $T_t^2$  is to be preferred when testing for genetic association SASIENI (1997).

For study of association between genotype and a quantitative trait, linear regression analysis is performed. Let us denote the vector of phenotypes as  $y$ , with particular values  $y_i$  ( $i = 1, \dots, N$ ). Let us code the genotypes with a quantitative variable, which reflects the number of  $B$  alleles. Thus, we will code  $AA$  as 0,  $AB$  as 1, and  $BB$  as 2. Let us denote the vector of genotypes as  $g$ , with particular values  $g_i$  ( $i = 1, \dots, N$ ) taking the value of 0, 1 or 2. The linear regression model assumes that the expectation of the trait is

$$E[y_i] = \mu + \beta_g \cdot g_i$$

where  $\mu$  is intercept and  $\beta_g$  is the coefficient of regression of the genotype on the phenotype.

The estimate of  $\beta_g$  is provided by a well-known expression

$$\hat{\beta} = \frac{Cov(y, g)}{Var(g)} = \frac{\sum x_i g_i / N - \sum x_i \sum g_i / N^2}{\sum g_i^2 / N - (\sum g_i)^2 / N^2} = \frac{N \sum x_i g_i - \sum x_i \sum g_i}{N \sum g_i^2 - (\sum g_i)^2} \quad (6.10)$$

Under the null hypothesis, the variance of  $\hat{\beta}$  is  $Var_0(\hat{\beta}_g) = \frac{Var(y)}{N \cdot Var(g)}$ , and the score test statistic

$$T_q^2 = \frac{\hat{\beta}_g^2}{Var_0(\hat{\beta}_g)} = \frac{Cov(y, g)^2}{Var(g)^2} \cdot \frac{N \cdot Var(g)}{Var(y)} = N \frac{Cov(y, g)^2}{Var(y) \cdot Var(g)} \quad (6.11)$$

is distributed as  $\chi^2$  with one degree of freedom. Here,  $Var_0 \hat{\beta}_g$  denotes the variance under the null hypothesis. Denote the correlation between  $y$  and  $g$ ,  $\frac{Cov(y,g)}{\sqrt{Var(y) \cdot Var(g)}}$ , as  $r$ . Then  $T_q^2 = N \cdot r^2$ .

It is worth to note that Armitage's trend test can be expressed as a linear regression test. In the table 6.5 Let us code the genotypic groups based on the number of  $B$  alleles. Thus, we will code  $AA$  as 0,  $AB$  as 1, and  $BB$  as 2. Let us also code the cases with "1" and controls with "0". Let  $y$  denote the vector of phenotypes and  $g$  the vector of genotypes coded in this way. Then, if we perform linear regression of the case-control status onto the number of  $B$  alleles in the genotype, the estimate of the regression coefficient is (following 6.10)

$$\hat{\beta} = \frac{\sum x_i g_i - \sum x_i \sum g_i / N}{\sum g_i^2 - (\sum g_i)^2 / N} = \frac{(r_1 + 2r_2) - \bar{p}R}{(n_1 + 4n_2) - \bar{p}N}$$

Thus, the expected proportions in the Armitage's trend test are provided by solution of linear regression equation, in which regression of case-control status, coded as "0" and "1" is performed onto the number of  $B$  alleles. It can be further demonstrated that the trend test statistics 6.9 can be expressed as  $T_t^2 = N \cdot r^2$ , where  $r^2$  is squared coefficient of correlation between  $y$  and  $g$ .

In the next two sections we will consider the effects of genetic structure on the standard tests for association described above. The extensive treatment of the problem is mainly due to the seminal works of Devlin, Roeder, Bacanu, and colleagues BACANU *et al.* (2000, 2002); DEVLIN and ROEDER (1999); DEVLIN *et al.* (2001); here, some of their results are repeated.

The genetic structure of the study population will be characterized by the kinship matrix – a square matrix, which, for all pairs of individuals in question, provides their pair-wise kinship coefficients (defined at page 140). The kinship coefficient between persons  $i$  and  $j$  will be defined as  $f_{ij}$ .

## 6.2.2 Effects of genetic structure on standard tests

As it was demonstrated above, the Armitage's trend test for association can be expressed as  $T_a^2 = \frac{\hat{\beta}^2}{Var(\hat{\beta})}$ . Genetic structure affects both numerator and denominator of this expression. Indeed, if measurements are dependent, the variance of the estimate is likely to be under-estimated if such dependence is not accounted for, leading to the inflation of the tests statistic. Secondly, even in absence of association, the estimate of the effect may be biased, again, leading to increased value of the test statistic.

Let us consider following artificial example. Let study cases are closely related individuals – sibs<sup>1</sup> – coming from one family, and study controls are sibs from other family. In essence, we compare the squared frequency difference between the two groups to the variance of this difference. If groups are formed by independent individuals, we can detect arbitrary small frequency differences by increasing the sample size, and consequently, decreasing the variance of the frequency estimate. This definitely is not the case when our study sample consist of a large sibship – whatever is the sibship size, only four alleles may be present in the parents, and the precision of the estimate of the allele frequency in the general population is limited – as if we had two people at maximum. If we do not

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<sup>1</sup>brothers and sisters



take this consideration into account, we are likely to over-estimate the precision of the frequency estimate; the denominator of the test statistic becomes small, and the statistic becomes large. Secondly, the parents of the "case" and "control" sibships have a high chance to be genotypically different, just by chance; and any genotypic configuration leading to the different number of  $B$  alleles will be reflected in difference in frequencies between sibships, given the sibships are large. Assuming HWE, the chance that two parental couples will have different number of  $B$  alleles is one minus the chance that they will have the same number of  $B$  alleles:

$$\begin{aligned} P(\#B_1 \neq \#B_2) &= 1 - (P(\#B_1 = \#B_2 = 0) + P(\#B_1 = \#B_2 = 1) + \dots + P(\#B_1 = \#B_2 = 4)) \\ &= 1 - (P(\#B_1 = 0)P(\#B_2 = 0) + \dots + P(\#B_1 = 4)P(\#B_2 = 4)) \\ &= 1 - (q^8 + 16p^2q^6 + 36p^4q^4 + 16p^6q^2 + p^8) \end{aligned}$$

which, for a common allele with frequency of 0.2 translates to the probability 0.64.

Let us quantify these two sources of bias. Following DEVLIN *et al.* (2001) let us assume that the number of cases and the number of controls is the same,  $N$ . Let us denote the vector of genotypes, coded as 0, 1, and 2 of cases as  $X$ , with  $X_i$  ( $i = 1, \dots, N$ ) being the genotype of the  $i$ -th case, and the vector of genotypes of controls as  $Y$  (with  $Y_j$ ,  $j = 1, \dots, N$  being the genotype of  $j$ -th control). The trend and the allelic tests statistic are proportional to the square of  $T = \sum X_i - \sum Y_j$ . The variance of this statistic, in general form, is

$$\begin{aligned} Var(T) &= \sum_{i=1}^N Var(X_i) + \sum_{i=1}^N Var(Y_i) \\ &\quad + 2 \sum_{i < j} Cov(X_i, X_j) + 2 \sum_{i < j} Cov(Y_i, Y_j) \\ &\quad - 2 \sum_i \sum_j Cov(X_i, Y_j) \end{aligned} \quad (6.12)$$

Let us consider a situation in which cases come from one population, and controls from the other population; each of the populations is in HWE and the difference between the populations is characterized with  $F_{st}$  (see equation 6.6). Under this model,  $Var(X_i) = Var(Y_i) = 2pq(1 + F_{st})$ , and the covariance between any pair of genotypes from the same population is  $4pqF_{st}$ . Then

$$\begin{aligned} Var(T) &= 2pq(1 + F_{st}) \cdot N + 2pq(1 + F_{st}) \cdot N \\ &\quad + 2 \cdot 2pqF_{st} \cdot N(N-1)/2 + 2 \cdot 2pqF_{st} \cdot N(N-1)/2 + 0 \\ &= 4pqN \cdot (1 + F_{st} + 2F_{st}(N-1)) \approx 4pqN(1 + 2NF_{st}) \end{aligned} \quad (6.13)$$

here,  $4pqN$  corresponds to the binomial variance of  $T$  in absence of genetic structure, while  $F_{st} + 2 \cdot F_{st} \cdot (N-1)$  reflects the inflation of the variance. As the second term is the function of the sample size, large inflation may be achieved even with small values of  $F_{st}$ . Note that here the sample size is  $2N$ , as we assumed  $N$  cases and  $N$  controls.

Above we have considered an example in which we know the  $F_{st}$  between the population of cases and the population of controls. In a practical study, a number of cases and controls is usually sampled from each genetically different population. Let the proportion of individuals sampled from population  $c$  among cases is  $a_c$  and the proportion of individuals coming from that population among controls is  $u_c$ . Then, it can be shown that

$$Var(T) \approx 4pqN(1 + 2 \cdot N \cdot F_{st} \cdot \sum_c (a_c - u_c)^2) \quad (6.14)$$

As it follows from this equation, the variance of the estimated frequency difference depends on the composition of the sample. Maximal inflation is achieved when the cases and controls are sampled from different populations, while if  $\sum_c (a_c - u_c)^2 = 0$  – which is achieved by sampling equal number of cases and controls from each sub-population – the variance inflation is minimal.

These results can be generalized to arbitrary relations between cases and controls. Let us denote kinship between cases  $i$  and  $j$  as  $f_{ij}^X$ , kinship between controls as  $f_{ij}^Y$ , and the kinship between a case and a control as  $f_{ij}^{XY}$ . Then DEVLIN *et al.* (2001)

$$\text{Var}(T) = 4pqN \cdot \left( 1 + F_{st} + \frac{2}{N} \sum_{i < j} f_{ij}^X + \frac{2}{N} \sum_{i < j} f_{ij}^Y - \frac{2}{N} \sum_i \sum_j f_{ij}^{XY} \right) \quad (6.15)$$

This equation demonstrates that the variance is inflated not only in a case when cases and controls come from genetically distinct populations, but that also "cryptic relations" among either cases (some  $f_{ij}^X > 0$ ) or controls (some  $f_{ij}^Y > 0$ ) may lead to increased variance of the test. Because summation (e.g.  $\sum_{i < j} f_{ij}^X$ ) is performed over all pairs of cases/controls, cryptic relations may have a strong impact onto inflation of the variance.

Generally, the variance of the  $T$  can be expressed as  $\text{Var}(T) = 4Npq(1 + F_{st} + D(f^X, f^Y, f^{XY}))$ . Here,  $4Npq$  corresponds to the binomial variance assuming HWE and independence between cases and controls, the second term –  $F_{st}$  – accounts for increase in variance due to deviation from HWE, and the last, which is a function of kinship –  $D(f^X, f^Y, f^{XY})$  – accounts for dependencies between cases and controls. The test  $\frac{T^2}{4Npq(1 + F_{st} + D(f^X, f^Y, f^{XY}))}$  is distributed as  $\chi^2$  with one degree of freedom.

The allelic and Armitage's trend statistics can be expressed as  $T_a^2 = \frac{T^2}{4Npq}$  and  $T_t^2 = \frac{T^2}{4Npq \cdot (1 + F_{st})}$ . What is their distribution when there is relatedness / stratification between cases and controls? Clearly, both of them are distributed as  $\tau^2 \cdot \chi^2$ , where  $\tau^2 > 1$  is the variance inflation factor. It is easy to show that it is equal to  $\tau^2 = 1 + F_{st} + D(f^X, f^Y, f^{XY})$  for the allelic and  $\tau^2 = \frac{1 + F_{st} + D(f^X, f^Y, f^{XY})}{1 + F_{st}}$  for the trend test.

In above, we have accounted for the over-dispersion of the test statistic due to possible relatedness between the cases and controls, and demonstrated that the allelic and the trend test are inflated by some constant  $\tau^2$ , which reflects the inflation of the variance,  $\text{Var}(T) = \sigma^2 \cdot \tau^2$ , where  $\sigma^2$  is the variance estimated under the assumption of independence. However, in the beginning of this section we have discussed another source of the inflation of the test statistic – the bias in frequencies between cases and controls due to confounding. Let cases and controls be sampled from different populations, whose difference is characterized by  $F_{st}$ , and let for some marker the allele frequency difference – which occurs entirely due to genetic structure of the samples – between cases and controls be  $d$ . The test statistic  $T$  is then distributed as Normal with mean  $Nd$  and variance computed in previous section,  $\sigma^2 \cdot \tau^2$ ,  $N(Nd, \sigma^2 \tau^2)$ . To address the issue of bias, we need to figure out the distribution of the mean –  $2Nd$ . In general, the expected frequency difference is zero – indeed, if we consider a large number of subpopulations, or a large number of markers, if allelic frequencies are determined by random effects, the deviation is likely to occur in any direction, resulting in zero on average. The variance of the  $d$  is

$2pqF_{st}$ , thus the variance of  $2Nd$  is  $(2N)^2 2pqF_{st}$ .<sup>2</sup> Thus finally,  $T$  is expected to be distributed as  $N(0, \sigma^2 \cdot \tau^2 + 8pqF_{st}N^2)$ . If we consider the variance of allelic test,  $\sigma^2 = 4pqN$ , then  $T \sim N(0, \sigma^2(\tau^2 + 2F_{st}N))$ , and, denoting  $2F_{st}N$  as  $\nu^2$ ,  $T \sim N(0, \sigma^2(\tau^2 + \nu^2))$ . Similar expression may be obtained for the Armitage's trend test.

Thus, in presence of genetic structure both allelic and the trend test are distributed as  $(\tau^2 + \nu^2) \cdot \chi^2$ . Note that while  $\sigma^2$  is a function of allelic frequency, the inflation factors  $\tau^2$  and  $\nu^2$  depend on the differentiation between the populations in question, as measured by  $F_{st}$ , sample size,  $N$ , and the composition of the case-control sample  $(a_c, u_c)$ , but does not depend on the allele frequency.

As it was mentioned earlier, the Armitage's trend test can be re-formulated as a regression-based score test, in which genotypes are coded as 0, 1, and 2, and phenotypes as 0 and 1. Therefore all above arguments apply to the analysis of quantitative traits as well. The test statistic  $T_q^2 = \frac{\hat{\beta}_g^2}{Var(\hat{\beta}_g)}$  in which  $\hat{\beta}$  and  $Var(\hat{\beta})$  are estimated using independence assumption is inflated because of over-dispersion ( $Var(\hat{\beta})$  is under-estimated if the relatedness is not accounted for), and also  $\hat{\beta}_g$  may be biased, because different genetic populations may well be characterized by different mean value of the trait (e.g. because of environmental influences). It can be shown that also for quantitative traits  $T_q^2$  is distributed as  $(\tau^2 + \nu^2) \cdot \chi^2$ , where  $\tau^2$  is inflation due to over-dispersion, and  $\nu^2$  is the inflation because of bias.

### 6.2.3 Genomic control

From previous section it follows that in presence of genetic structure the standard association statistics may be inflated and the distribution is described as  $(\tau^2 + \nu^2) \cdot \chi^2$ . Let us denote  $(\tau^2 + \nu^2)$  as  $\lambda$  for short.  $\lambda$  depends on the genetic structure of the sample, as characterized by pairwise kinship, and sample size,  $N$ . For binary trait analysis, it also depends on the composition of the case-control sample, as expressed by the proportion of cases/controls coming from particular population,  $c$  ( $a_c, u_c$ ). For quantitative trait, it depends of heritability of the trait, and environmentally determined differences co-occurring with the difference in kinship. However,  $\lambda$  does not depend on the allele frequency. Therefore, for any particular study sample, if  $F_{st}$  is constant over the genome,  $\lambda$  is also a constant.

Therefore  $\lambda$  can be estimated from the genomic data, using "null" loci – a set of random markers, which are believed not to be associated with the trait. This estimate can then be used to correct the values of the test statistic at the tested loci – a procedure, known as "genomic control". The test statistics computed from these loci thus estimates the distribution of the test statistic under the null hypothesis of no association. Let us consider  $M$  "null" markers, and denote the test statistic obtained from  $i$ -th marker as  $T_i^2$ . Given genetic structure determines inflation  $\lambda$ ,  $T^2 \sim \lambda \cdot \chi_1^2$ . The mean of a random variable coming from  $\chi_1^2$  is equal to 1; if a random variable comes from  $\lambda \cdot \chi_1^2$ , the mean would be  $\lambda$ . Thus we can estimate  $\lambda$  as the mean of the obtained "null" tests. In practice, it is recommended to use the ratio between the observed median

<sup>2</sup> For the case when some proportion,  $a_c$ , among cases comes from population  $c$ , and some proportion of controls,  $u_c$ , comes from population  $c$   $Var(d) = 2pqF_{st} \sum_c (a_c - u_c)^2$

and the one expected for  $\chi_1^2$ :

$$\hat{\lambda} = \frac{\text{Median}(T_i^2)}{0.4549} \quad (6.16)$$

For the tested markers, the corrected value of the test statistic is obtained by simple division of the original test statistic value on  $\hat{\lambda}$ ,  $T_{corrected}^2 = T_{original}^2 / \hat{\lambda}$ . Note that this procedure is correct only if the same number of study participants was typed for any marker, as you will see later.

As you can see, the genomic control procedure is computationally extremely simple – one needs to compute the test statistic using a simple test (e.g. score test), compute the median to estimate  $\lambda$ , and divide the original test statistics values onto  $\hat{\lambda}$ .

How to choose "null loci" is a GWA study? In a genome, we expect that a small proportion of markers is truly associated with the trait. Therefore in practice, all loci are used to estimate  $\lambda$ . Of course, if very strong (or multiple weak) true associations are present, true association will increase the average value of the test, and genomic control correction will be conservative. To relax this, it has been suggested SLADEK *et al.* (2007) to use, say, 95% of the least significant associations for estimation of the inflation factor; however, the question of selection of cut-off is not obvious, and all markers are used in practice in most studies.

We have observed that the inflation factor  $\lambda$  is a function of sample size,  $N$  – the bigger is the sample size, the larger is inflation. If this is the case, how can we compare inflations between different GWA studies? A good idea is to use a standardized inflation, say, inflation per 1,000 subjects. For a quantitative trait analysis, inflation factor can be expressed as  $\lambda \approx (1 + N * D(sample))$ , where  $N * D(sample)$  is a term, which grows linear with sample size, at some rate determined by sample characteristics.

Therefore a standarsized inflation factor can be etimated as

$$\hat{\lambda}_{1000} = 1 + \frac{\hat{\lambda} - 1}{N} \cdot 1000,$$

where  $N$  is the sample size and  $\hat{\lambda}$  is the estimate from the total sample.

What about a case-control sample? For such samples, a standardized  $\lambda$  is computed for a fixed number of cases and controls, say 1000; we will denote such standardized inflation as  $\lambda_{1000,1000}$  to distinguish it from the one computed for a quantitative traits. If we denote the number of cases as  $N_a$  and the number of controls as  $N_u$ , then

$$\hat{\lambda}_{1000,1000} = 1 + \frac{500 \cdot (\hat{\lambda} - 1) \cdot (N_a + N_u)}{N_a \cdot N_u}$$

Clearly, we have assumed that in our GWA study the sample size was equal for all studied markers; this fact allows for very simple estimation of  $\hat{\lambda}$  (equation 6.16) and correction of the test statistic. It may happen, however, that the number of participants typed for different marker loci is different; for example, this may happen when all study participants were typed for SNP panel one, and then a part of the study was additionally typed at a different panel. In such

situation, expectation of a  $T_q^2$  test for a quantitative trait can be expressed as

$$E[T_q^2] = 1 + (\lambda_{1000} - 1) \cdot \frac{N}{1000}$$

It is straightforward to obtain an estimate of  $\lambda_{1000}$  by performing linear regression of the observed test statistic values onto the sample size used; note that the intercept should be fixed to 1 in this procedure. Other, more effective procedures may be thought of.

For binary traits, if sample is composed from  $N_a$  cases and  $N_u$  controls, the expression for the expected value of the test statistic is similar to that obtained for quantitative traits, however, geometric mean of the number of cases and controls are used FREEDMAN *et al.* (2004):

$$E[T_t^2] = 1 + (\lambda_{1000,1000} - 1) \cdot \frac{\frac{1}{1000} + \frac{1}{1000}}{\frac{1}{N_a} + \frac{1}{N_u}}$$

This relation can be used to estimate  $\lambda_{1000,1000}$ .

We have considered the genomic control procedure for the tests assuming additive effects of the locus onto phenotype. Under this model, inflation of the tests ( $\lambda$ ) does not depend on allelic frequency, which allows a straightforward estimation of  $\lambda$  and further correction of the tests. Does the same apply to other genetic models?

In figure 6.4 the results for additive, dominant, recessive, and over-dominant model tests are presented. Simulated study consisted of 1000 cases and 1000 controls; the differentiation between the case and control populations was assumed  $F_{st} = 0.002$ . Armitage's trend test was used in analysis. One can see that when a common polymorphism is studied ( $0.05 < q < 0.95$ ), the additive model test (green line) does not depend on the frequency of the  $B$ . The range of allele frequencies in which the additive model does not depend on these depends of the values of  $F_{st}$  and the sample size: the larger differentiation, and the smaller the sample size, the narrower is the range.

However, other one degree of freedom tests (recessive, red; dominant, blue; over-dominant, cyan) do depend on the allele frequency very much across all the range of allelic frequencies (figure 6.4, see ZHENG *et al.* (2005) for details).

Thus, it is important to remember that the genomic control was developed for, and works with additive genetic models; even in this framework, behaviour of  $\lambda$  depends on allelic frequencies when these are too low or too high – thus application of genome-wide derived inflation factor to such marker loci may be incorrect for loci with low minor allele frequency. What is somewhat sustaining statistically is the fact that such correction will be conservative, and not liberal – but the same may be worrisome biologically (e.g. missed true positives). For other types of model,  $\lambda$  can, at least in theory, be estimated taking into account the allelic frequency of the locus in question. However, these methods are not implemented yet in packages for genome-wide association analyses.

Other important thing to remember about genomic control is that it assumes uniform  $F_{st}$  across the genome. This may not be the case for some (e.g. selected) genomic regions. Such regions may still generate very high test statistic, even after appropriate genomic control correction CAMPBELL *et al.* (2005); basically, the frequency distribution at such loci may vary from population to population much more than the average across the genome. A number of methods

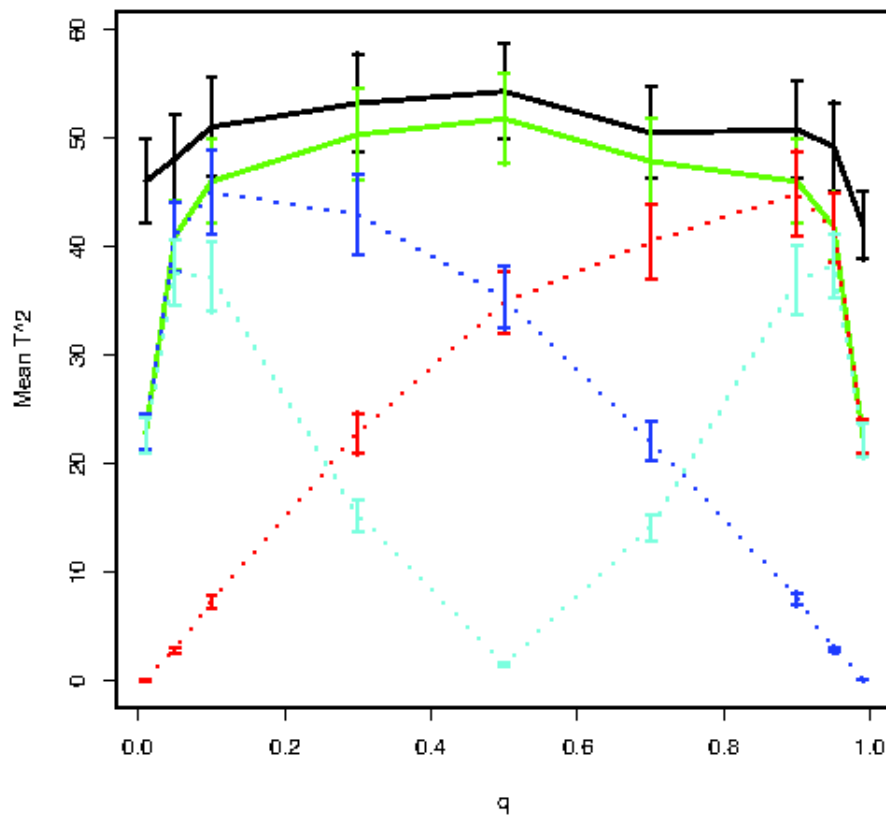


Figure 6.4: Mean  $T_t^2$  test statistic as frequency of the  $B$  allele,  $q$ . Models considered: additive, green; recessive, red; dominant, blue; over-dominant, cyan.

taking genetic structure of study population into account directly (structured association, EIGENSTRAT, reviewed later), can deal with such situations.

Finally, what levels of genomic control inflation parameter are acceptable in GWA studies? As the question is of how much the total test statistic is affected, non-standardized inflation may be used to address the question. Despite of lack of clear guidance, the general practice in the field is to consider the values of  $\hat{\lambda} < 1.01$  as small,  $\hat{\lambda} < 1.05$  as moderate and still acceptable. If  $\hat{\lambda} > 1.1$ , this suggest strong influence of genetic structure or other design factors on the test statistic. While GC statistically correct method, such analysis lacks power; good practice is to consider use of other methods, which take the structure of the sample into account in direct manner.

### 6.3 Analysis of structured populations

A study population may be structured in at least two ways. Firstly, while study participants may be "independent" in that kinship between them is expected to be very low (e.g. random sample from a large outbred population), they may belong to rather different genetic populations. An extreme example of such study is a study aiming to combine the data from a sample obtained by random ascertainment from the population of Beijing and a sample from Amsterdam. While in any of the samples people are only remotely related ("independent"), the two samples in question are characterized by high genetic differences; also the environmental influences may be very different for the two populations. The distribution of the trait under the study may be essentially different between the two populations: not only the mean values, but also variances.

On the other hand, consider a family-based sample from genetically isolated population. Here, environmental influences are more or less uniform for all study participants; the distribution of the trait may be assumed to be governed by the same set of rules for all study participants. However, they are characterized by high and variable kinship between each other.

These two flavours of stratification may be expressed in terms of kinship. When we talk about different "populations", we assume very low kinship and high genetic differentiation between the members of these; when we talk about "relatedness", we assume high kinship and small degree of differentiation. This is illustrated at figure 6.5. Roughly speaking, when we expect that study participants share common ancestors few (1-4) generation ago, the kinship between study participants is high, and the study can be classified as a family-based one (Study 1 of figure 6.5). When common ancestors between study participants is expected say >5 generations ago, the kinship between study participants is low, and such study may be classified as a population-based sample of "independent" people (Studies 2 and 3 of figure 6.5). Now consider two groups of study participants (samples 2 and 3). Any person from group 2 is expected to share a common ancestor with a person from the same study group with much higher probability than with a person from group 3; thus these represent two genetic populations (see page 141 for the retrospective definition of genetic population). It should be kept in mind that if kinship is very low (expected common ancestors dozens of generations ago), this would translate in high degree of genetic differentiation. Moreover, this reflects a long history of isolation, which usually means geographic separation, and accumulation of cultural and other environ-

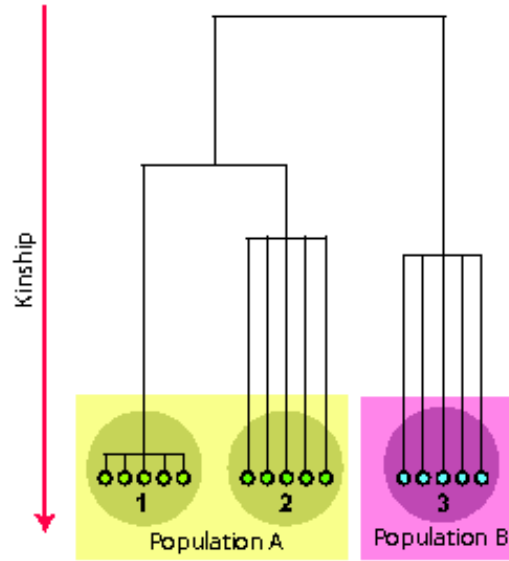


Figure 6.5: Three samples from two populations. 1: Family-based sample; 2,3: random sample of "independent" people

mentsl differences between the populations, which may be crucial in association studies.

Of cause, a particular study is usually characterized by some mixture of populations and some relatednes between study participants. We will, however, first consider the two extreme scenarios – analysis of samples of "independent" subjects from different populations, and analysis of a family-based study.

### 6.3.1 Structured association

If study populayion consist of a mixture of several distinct genetic populayions, and, within each sub-population, the study participants are remotely related, structured asoiation analysis may be the method of choice. In such analysis, effect and its variance are estimated within each strata separately, and then these estimates are pooled to generate global statistics. The strata can be known from design (e.g. place of birth or ethnicity of parents) or estimated from GWA data. By doing this, we allow for arbitrary trait distribution, characterized by stratum-specific mean and variance, in each stratum. Clearly, this may be crucial when different populations characterized by different environments are included in analysis.

Combining the evidence across strata may be done using a number of methods, e.g. Cochran-Mantel-Haenszel test for binary outcomes. One of the most simple ways to combine the evidences coming from multiple strata – or studies – is to use fixed effects inverse variance meta-analysis.

In essence, this method is equivalent to combining likelihoods coming from separate studies, using quadratic approximation. Denote coefficients of regression estimated in  $N$  studies/strata as  $\beta_i$ , and associated squared standard errors



of the estimates as  $s_i^2$  where  $i \in 1, 2, \dots, N$ . Note that the regression coefficient should be reported on the same scale, e.g. centimeters, meters, or using observations reported on the standard normal scale. Define weights for individual studies as

$$w_i = \frac{1}{s_i^2}$$

Then the pooled estimate of the regression coefficient is

$$\beta = \frac{\sum_{i=1}^N w_i \beta_i}{\sum_{i=1}^N w_i}$$

As you can see, the weights have straightforward interpretation: the bigger the weight of the study (meaning the smaller is the standard error in the study), the larger is the contribution from this study onto the pooled estimate.

The standard error of the pooled estimate is computed as

$$s^2 = \frac{1}{\sum_{i=1}^N w_i}$$

and the  $\chi^2$ -test for association is computed in standard manner as

$$T^2 = \frac{\beta^2}{s^2} = \frac{\left(\sum_{i=1}^N w_i \beta_i\right)^2}{\sum_{i=1}^N w_i}$$

or, alternatively, the Z-test is

$$Z = \frac{\beta}{s} = \frac{\sum_{i=1}^N w_i \beta_i}{\sqrt{\sum_{i=1}^N w_i}}$$

When binary traits are studied, and results are expressed as Odds Ratios with  $P$  – values, it is also possible to apply inverse variance method. For this, you need to transform your Odds Ratios using natural logarithm, and, on this scale, estimate the standard error. Generic inverse variance pooling may be applied to the data transformed this way; the final results are back-transformed onto Odds Ratio scale using exponentiation.

In meta-analysis procedure, it is assumed that study populations are composed of genetically homogenous "unrelated" individuals. Each study is first analysed separately, and genomic control should either show no inflation of the test statistic, or a small one, which should be corrected with GC prior to combining of strata/studies. If some of the strata demonstrate large residual inflation, this may suggest further sub-structure present in that strata – it should be dealt with using further sub-division and structure association, or methods described further in this chapter, prior to attempting the pooling of results.

The meta-analytic methodology to perform structured association analysis outlined above has advantage of simplicity. However, it assumes goodness of quadratic approximation of the likelihood function, which is achieved if numbers are large. If numbers are not large (e.g. rare polymorphisms), a more complicated strategy can be used. A regression model, allowing for study-specific mean, effects of nuisance parameters, and residual variances, but unique (across the studies) effect of interest may be formulated. Analysis of such model is equivalent to the above-described meta-analysis procedure, under large numbers assumption.

### 6.3.2 Mixed models based approach

In this section we will consider the methods used to analyse samples in which close family relations may be present; these may be family-based samples from outbred populations, or random samples from genetically isolated populations, where, due to limited population size, relatives are likely to be present by chance.

The mixed model based ideology stems from the classical animal breeding and human heritability analysis methodology, dating back to works of Fisher (1918). He proposed a model in which a very large number of Mendelian genes contribute small effects to the phenotype (infinitesimal model). We may express this model as

$$y_i = \mu + G_i + e_i \quad (6.17)$$

– the value of the trait of  $i$ -th person is sum of grand population mean, contribution from multiple additively acting genes of small effect ( $G_i$ ), and residual error ( $e_i$ ).

Under this assumption, the distribution of the trait in a pedigree is described by a multivariate normal distribution with number of dimensions equal to the number of phenotyped people, with the expectation of the trait value for some individual  $i$  equal to

$$E[y_i] = \mu$$

where  $\mu$  is grand population mean (intercept). The variance-covariance matrix is defined through its elements  $V_{ij}$  – covariance between the phenotypes of person  $i$  and person  $j$ :

$$V_{ij} = \begin{cases} \sigma_e^2 + \sigma_G^2 & \text{if } i = j \\ 2 \cdot f_{ij} \cdot \sigma_G^2 & \text{if } i \neq j \end{cases} \quad (6.18)$$

where  $\sigma_G^2$  is variance due genes,  $f_{ij}$  is kinship between persons  $i$  and  $j$ , and  $\sigma_e^2$  is the residual variance. The proportion of variance explainable by the additive genetic effects,

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_e^2}$$

is termed (narrow-sense) "heritability".

Fixed effects of some factor, e.g. SNP, may be included into the model by modifying the expression for the expectation, e.g.

$$E[y_i] = \mu + \beta_g \cdot g$$

leading to so-called "measured genotypes" model (BOERWINKLE *et al.* (1986).

In essence, this is a linear mixed effects model – the one containing both fixed (e.g. SNP) and random (polygenic) effects. A large body of literature is dedicated to this class of models, and finding the solution for such regression represents no great methodological challenge. Applicability of this model to GWA analysis was first proposed by Yu and colleagues (YU *et al.* (2006)). Two problems occur with application of this model to GWA analysis: firstly, if standard Maximum Likelihood (or Restricted Maximum Likelihood) approach is used to estimate this model, the computational time is excessive (AULCHENKO *et al.* (2007a)); secondly, the pedigree – and thus the kinship matrix – may be not known for study participant.

Let us first consider existing solutions to the first problem. High computational complexity occurs if both random and fixed effects part of the model

are estimated simultaneously, and this procedure is repeated for every SNP analysed. However, we may assume that the effects of SNP are likely to be small. Thus, inclusion of the SNP into the model is not likely to change the estimates of the components of variance. Therefore a two-step procedure was suggested AULCHENKO and STRUCHALIN (submitted); CHEN and ABECASIS (2007): firstly, the mixed model containing all terms but these involving SNPs, is estimated (this includes estimation of the components of variance, and possibly the nuisance fixed effects); secondly, these estimates are then used to compute a test for association for every SNP in analysis. For details on this test, see CHEN and ABECASIS (2007) for original formulation, and AULCHENKO and STRUCHALIN (submitted) for extension to multiple variables and interaction effects tested in step two. The model is implemented in MACH and ProbABEL software packages.

Another method, bearing close resemblance to the method described above, is GRAMMAR – Genome-wide Rapid Analysis using Mixed Models and Regression AULCHENKO *et al.* (2007a). Here, in step one, not only the mixed model is estimated, but also the environmental residuals,

$$\hat{e}_i = y_i - (\hat{\mu} + \hat{G}_i)$$

are estimated. These are free of correlations, and can be used as the new "trait" in further analysis. This procedure allows for very fast GWA analysis in step 2 (for large sample sizes, much faster than above-described two-step approach), also it has a great flexibility as the environmental residuals can be analysed using a large variety of methods. At the same time, the GRAMMAR test for association is conservative, and the estimates of the effects obtained in step 2 are downward biased AULCHENKO *et al.* (2007a). While conservativity of the test may be dealt with using "reverse genomic control" AMIN *et al.* (2007), the issue of bias in effect estimates has no solution yet. The GRAMMAR model is implemented in GenABEL software package AULCHENKO *et al.* (2007b).

Strictly speaking, above described two-step tests are correct if the distributions of covariates in the first and the second parts of the model are independent conditional on the estimated phenotypic variance-covariance matrix. This assumption is most likely to be true when the covariates included in the base model are environmental ones, and thus are not expected to exhibit conditional correlation with SNPs. However, when endogenous risk factors, such as e.g. body mass index, are included as the covariates in the base model, some SNPs are expected to exhibit covariance with this covariate. In such situation the two-step test is not strictly correct, but given relatively weak SNP-phenotype correlations normally observed in GWAS should normally keep good statistical properties in most situations. The properties of the test under such conditions are still awaiting their description.

The methodology described here applies to study of association of quantitative traits. For binary traits, no formal practical GWA solution is available yet. The best current strategy to analyze binary traits in samples of relatives may be to treat the binary outcome as if it was quantitative. As you have seen with Armitage's trend test, which is equivalent to the score test for a quantitative variable when outcome is coded with "0" and "1" (page 152), this approach leads to correct inferences about significance.

### 6.3.3 Estimation of kinship matrix from genomic data

Another problem with application of mixed models to analysis of GWA data is that pedigree is required to estimate the kinship matrix. The expectation of kinship can be estimated from pedigree data using standard methods, for example the kinship for two outbred sibs is  $1/4$ , for grandchild-grandparent is  $1/8$ , etc. For an outbred person, the kinship coefficient is  $1/2$  – that is two gametes sampled from this person at random are IBD only if the same gamete is sampled. However, in many situations, pedigree information may be absent, incomplete, or not reliable. Moreover, the estimates obtained using pedigree data reflect the expectation of kinship, while the true realization of kinship may vary around this expectation. In presence of genomic data it may therefore be desirable to estimate the kinship coefficient from these, and not from pedigree. It can be demonstrated that unbiased and positive semi-definite estimator of the kinship matrix ASTLE and BALDING ((in press)) can be obtained by computing the kinship coefficients between individuals  $i$  and  $j$  with

$$\hat{f}_{ij} = \frac{1}{L} \sum_{l=1}^L \frac{(g_{l,i} - p_l)(g_{l,j} - p_l)}{p_l(1 - p_l)} \quad (6.19)$$

where  $L$  is the number of loci,  $p_l$  is the allelic frequency at  $l$ -th locus and  $g_{l,j}$  is the genotype of  $j$ -th person at the  $l$ -th locus, coded as 0,  $1/2$ , and 1, corresponding to the homozygous, heterozygous, and other type of homozygous genotype. AMIN *et al.* (2007); ASTLE and BALDING ((in press)); PRICE *et al.* (2006) The frequency is computed for the allele which, when homozygous, corresponds to the genotype coded as "1".

Interestingly, use of the kinship matrix instead of pedigree kinship (when available) may lead to higher power, especially when "dense" pedigrees and traits with high heritability are considered (YSA, unpublished data). This is likely to happen because genomic-based kinship is likely to reflect true genetic relations better than (possibly not completely correct) pedigree expectations.

### 6.3.4 EIGENSTRAT and related methods

EIGENSTRAT PRICE *et al.* (2006) method combines the ideas underlying structured association and the genomic kinship matrix. Basically, it allows for structured association, with strata and more subtle structure identified through the use of genomic data.

The genomic kinship matrix is computed as described earlier (equation 6.19). This matrix reflects genetic similarities between study participants. A reverse metric, distance matrix, is computed as  $0.5 - f$ . Classical multi-dimensional scaling (CMDS) is applied to identify a number (say,  $k$ ) of first principal components (PCs) of variation of the distance matrix. The idea behind CMDS is mapping of the all pair-wise distances defined by the original matrix to some  $k$ -dimensional space. Each study participant is presented as a dot in this space, and these dots are constructed in such way, that the distance between these is maximally close to the distances observed in the original distance matrix. Thus, if two genetically distinct populations are present in a sample, the first principal axis of variation ( $k = 1$ ) will identify these. Generally, of  $n$  distinct populations are present, these can be identified by  $k = n - 1$  first axes.

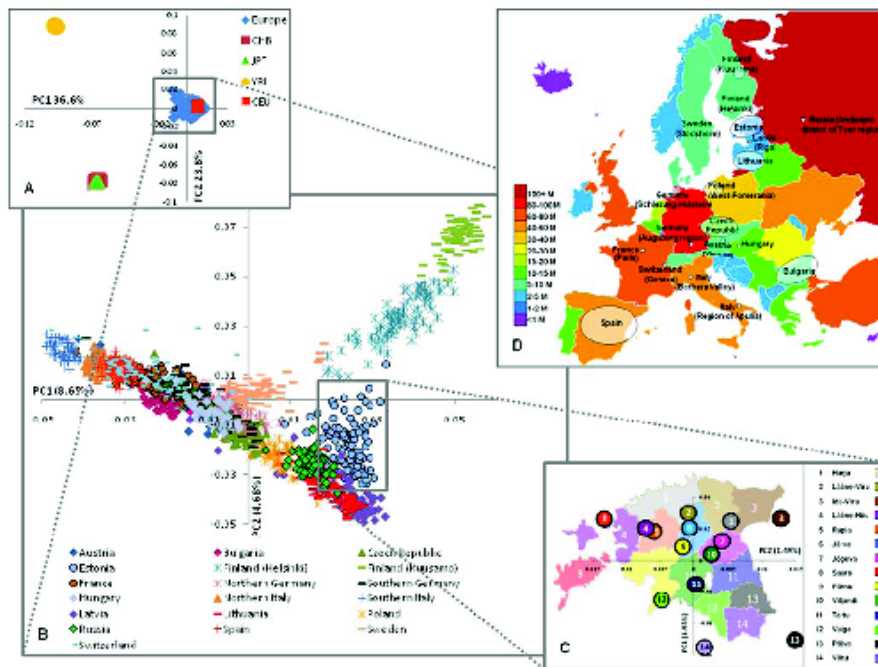


Figure 6.6: The European genetic structure (based on 273,464 SNPs). Three levels of structure as revealed by PC analysis are shown: A) inter-continental; B) intra-continental; and C) inside a single country (Estonia), where median values of the PC1&2 are shown. D) European map illustrating the origin of sample and population size. CEU – Utah residents with ancestry from Northern and Western Europe, CHB – Han Chinese from Beijing, JPT – Japanese from Tokyo, and YRI – Yoruba from Ibadan, Nigeria. (reproduced from NELIS *et al.* (2009))  
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To demonstrate this principle, results of CMDS of the genomic distance matrix for the HapMap participants, and a number of people from Europe, are presented at figure 6.6. On panel **A**, the results of CMDS of the distance matrix to the space of the first two PCs are present. Inter-continental differences are obvious – there are three distinct clusters, corresponding to Yoruba (yellow-brown), Asian (green and dark brown), and European (blue and red) study participant. You can see that mapping to the first two principal components perfectly distinguishes these three populations. The same methodology can be used to distinguish between more subtly divided populations – at panel **B** of figure 6.6 the mapping of European populations is present. The panel **C** presents even further detailisation of genetic distances – to the level of single country.

Note that configuration of European cluster in panel **A** is not exactly the same as in panel **B**. This happens because the distance matrix, computed based on formula 6.19, depends on allelic frequencies, as estimated in the total sample. Clearly, these are different, when all continents or Europeans only are used. Consecutive analysis of sub-samples allows distinguishing more and more subtle differences between populations.

Interestingly, the genetic distances co-incide very well with geographic distances, i.e. the populations close geographically are also close genetically. Thus, most isolation between human populations is explained by geographic distance.

How these principal components may be incorporated in association analysis? Different populations may have different mean values (or prevalences) of the trait under the study, which, coupled with genetic differences, leads to confounding and false positives in association study. A simple method would be to try to account for these differences in the means by incorporating the principal components as covariates into association analysis (SLADEK *et al.* (2007)). Another, related method would be to perform linear adjustment of both the phenotype and the genotype onto PCs (i.e. compute the residuals from linear regression of genotypes/phenotypes onto PCs). Then simple score test for association ( $T_q^2$ , equation 6.11) can be used to analyze association. This comprises the original EIGENSTRAT methodology suggested by Price and colleagues (PRICE *et al.* (2006)).

A practically interesting question is how many PCs to include into analysis? By default, EIGENSTRAT uses 10 PCs, but this is rather arbitrary number. In general, "significant" PCs should be included into analysis. Patterson and colleagues (PATTERSON *et al.* (2006)) suggested including the leading PCs, which significantly explain progressively more genetic variation. Alternatively, it was argued that PCs significantly associated with the phenotype should be included (NOVEMBRE and STEPHENS (2008)), as inclusion of other PCs is not only unnecessary, but may also reduce power.

Note that methods described here allow for differences in means between the populations, but not for the difference in variances. It would be interesting to address the question what effects violation of this assumption may have on association studies, and develop extended methods allowing for difference in variances. This would first probably require a study of inter-populational variances.

### 6.3.5 Summary: what method to use?

What methods should be used for analysis of particular study? An simplified overview is provided at figure 6.7.

Basically, if study participants come from the same genetic population, are characterized by low degree of kinship, and confounding, as measured with genomic control (GC) inflation parameter  $\lambda$  is small, GC is enough to correct for residual bias.

If study participants come from substantially different populations, and, for each population, above conditions hold, structured association may be used.

If study participants are characterized by high degree of kinship, mixed-model based methods should be used.

EIGENSTRAT and PC-based adjustment methods should be used in somewhat intermediate situation when differentiation between the populations and kinship between study participants is not too high: while substantial population differentiation may mean differences in variances, which EIGENSTRAT/PCBA does not account for, it is also known that these methods do not perform very well in pedigrees (YSA, Najaf Amin, unpublished data).

Clearly, a particular study may have its own specifics and should be considered separately. As difference between outlined scenarios is quantitative rather

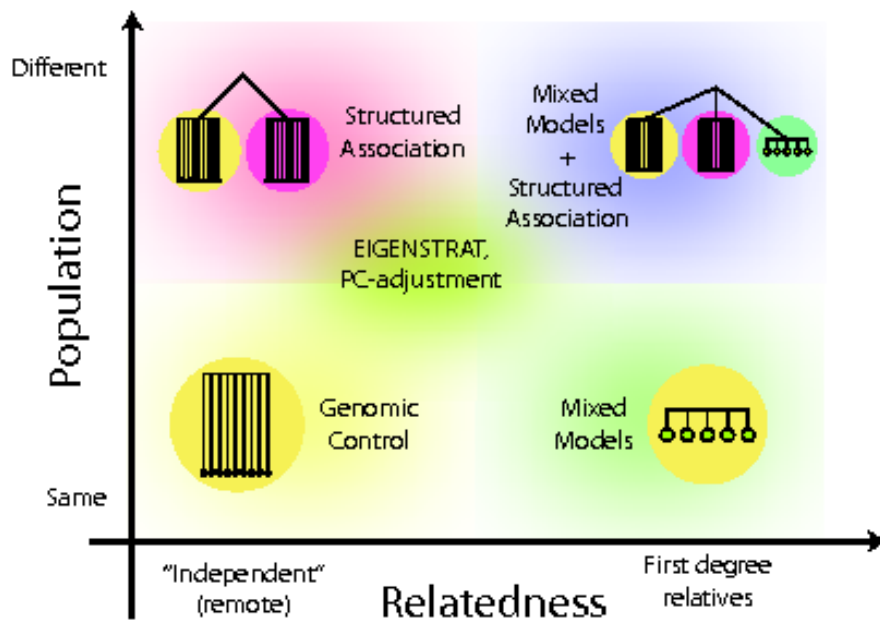


Figure 6.7: Applicability of different methods for association analysis.

than qualitative, there is no single recipe for analysis when genetic structure is present in a study sample.

## 6.4 Links

Here are some useful links to software which can be used for analysis in structured populations:

**EIGENSTRAT:** <http://genepath.med.harvard.edu/~reich/EIGENSTRAT.htm>

**GenABEL:** <http://mga.bionet.nsc.ru/~yurii/ABEL/>

**MACH:** <http://www.sph.umich.edu/csg/abecasis/mach/>

**PLINK:** <http://pngu.mgh.harvard.edu/~purcell/plink/>





## Chapter 7

# GWA in presence of genetic stratification: practice

Both ethnic admixture and presence of close relationships represents examples of confounding in association analysis. However, the methods to correct for stratification as resulting from mixture of subjects coming from different genetic populations, and methods to correct for family relations may be slightly different, and will be described separately in the next sections.

### 7.1 Analysis with ethnic admixture

In previous section we detected genetic stratification by analysis of genomic kinship matrix and excluded genetic outliers from our further analysis. When there are only a few such outliers, exclusion them from analysis is a good option. However, in large studies cases and controls are usually selected across a number of locations and genetic populations, and stratification is expected by design. In such case, analysis of association should account for this stratification.

Let us do structured association analysis using the `data1` data derived in previous section.

If you are not running R yet, start R and load `GenABEL`-package library by typing

```
> library(GenABEL)
```

and load the 'data1' workspace generated in section 5 ("Genome-wide association analysis").

```
> load("data1.RData")
> ls()
```

```
[1] "c11"          "data1"        "data1.gkin"  "data2.qt"    "old"
```

First, let us check how much test statistic inflation is there if we ignore stratification.

```
> data1.qt <- qtscore(dm2,data1)
> lambda(data1.qt)
```

```

$estimate
[1] 1.051744

$se
[1] 0.000756613

$iz0
[1] 1.015643

$iz2
[1] 1

```

We now will consider several ways to account for stratification, namely, structured association analysis, method of Price et al. (EIGENSTRAT), a similar method based on adjusting for the principal components of variation of genomic kinship matrix, and use of a mixed model.

One of the ways to do that is to perform *structured association* analysis. In such analysis, effect and its variance are estimated within each strata separately, and then these estimates are pooled to generate global statistics. The strata can be known from design (e.g. place of birth or ethnicity of parents) or estimated from GWA data.

To do structured association analysis we need to define a variable which will tell what population the study subjects belong to. In previous section, we stored the names of 'outlier' subjects in variable `c11`:

```

> c11

[1] "id2097" "id6954" "id2136" "id858"

```

We can use function `%in%` to find out what names of subjects are in `c11`:

```

> pop <- as.numeric(idnames(data1) %in% c11)

```

Let us check how the 'population' is distributed among the cases and controls:

```

> table(pop, phdata(data1)$dm2)

pop  0  1
  0 47 77
  1  0  4

```

As we have seen before, one of the clusters contains only the cases.

Now, structured association may be done with `qttscore` function by specifying `strata` argument:

```

> data1.sa <- qttscore(dm2, data=data1, strata=pop)
> lambda(data1.sa)

$estimate
[1] 1.03431

$se

```

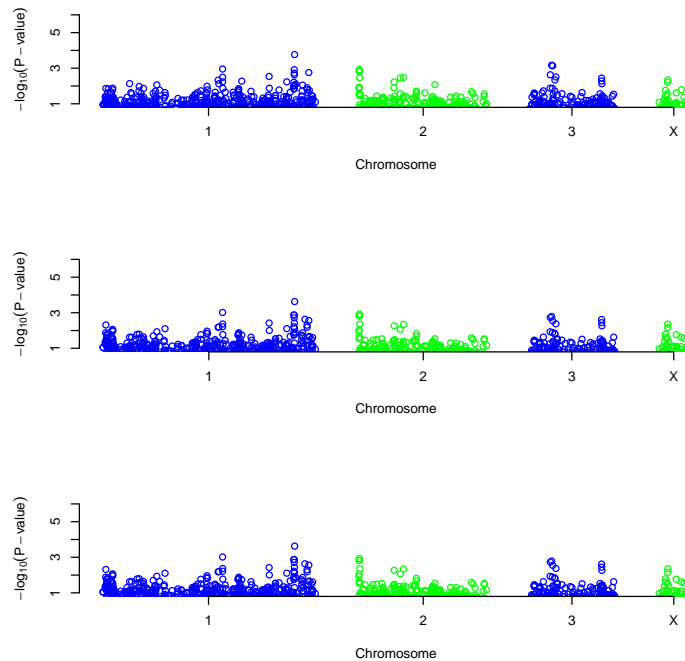


Figure 7.1: Comparison of the original results, results of analysis excluding outliers, and structured association analysis.

```
[1] 0.0007059588
```

```
$iz0
```

```
[1] 1.006671
```

```
$iz2
```

```
[1] 1
```

We can compare the original results, results of analysis excluding outliers, and structured association analysis by

```
> par(mfcol=c(3,1))
> plot(data1.qt,ylim=c(1,6))
> plot(data2.qt,ylim=c(1,6))
> plot(data1.sa,ylim=c(1,6))
> par(mfcol=c(1,1))
```

The resulting plot is presented at figure 7.1. In this case, there is little difference, because all people belonging to the smaller sub-population are cases.

Other way to adjust for genetic (sub)structure is to apply the method of Price et al. (*EIGENSTRAT*), which make use of principal components of the genomic kinship matrix to adjust both phenotypes and genotypes for possible stratification. In **GenABEL**-package, such analysis is done using **egscore** function.

```
> data1.eg <- egsscore(dm2,data=data1,kin=data1.gkin)
> lambda(data1.eg)
```

```
$estimate
[1] 1.102747
```

```
$se
[1] 0.001043038
```

The analysis plot may be added to the previous one by

```
> par(mfcol=c(3,1))
> plot(data1.eg,ylim=c(1,6))
```

Now let us apply *adjustment for the stratification by use of the principal components of genetic variation*. For that we first need to extract the principal components of genetic variation by constructing the distance matrix

```
> dst <- as.dist(0.5-data1.gkin)
```

and performing the classical multidimensional scaling

```
> pcs <- cmdscale(dst,k=10)
> pcs[1:5,]
```

	[,1]	[,2]	[,3]	[,4]	[,5]
id199	0.030289769	0.057952824	0.10342206	-0.020496319	-0.02678162
id300	0.031798583	0.004789923	0.02947431	0.033227508	0.05977593
id403	0.060654557	0.079249013	-0.06111279	-0.081417523	0.03294180
id415	-0.004913697	-0.030714900	0.07807686	0.017468144	0.03078079
id666	-0.012639670	0.016176179	-0.08784632	-0.002322116	0.02957059
	[,6]	[,7]	[,8]	[,9]	[,10]
id199	0.08021104	0.01144582	-0.020228061	-0.001507913	-0.060483058
id300	0.03702791	0.02887831	0.005142389	-0.005340720	0.094991803
id403	-0.01481834	-0.01259170	0.040037267	-0.048689378	0.007304146
id415	-0.02472941	0.07900292	0.075300986	0.028609310	0.021747288
id666	-0.04742543	-0.11594090	0.106539735	0.026380262	-0.022472669

Now we can use these PCs for adjustment:

```
> data1.pca <- qtsscore(dm2~pcs[,1]+pcs[,2]+pcs[,3],data1)
> lambda(data1.pca)
```

```
$estimate
[1] 1.004946
```

```
$se
[1] 0.0007356663
```

```
$iz0
[1] 1
```

```
$iz2
[1] 1
```

```
> plot(data1.pca,ylim=c(1,6))
```

Finally, let us use the full genomic kinship matrix for the adjustment for populational structure. First, let us estimate the polygenic model with

```
> h2a <- polygenic(dm2,data1,kin=data1.gkin)
```

The resulting 'heritability' estimate is

```
> h2a$esth2
```

```
[1] 0.3840789
```

Now we can perform mixed model approximation analysis using `mm score` function

```
> data1.mm <- mmscore(h2a,data1)
> lambda(data1.mm)
```

```
$estimate
```

```
[1] 1
```

```
$se
```

```
[1] NA
```

```
> plot(data1.mm,ylim=c(1,6))
```

```
> par(mfcol=c(1,1))
```

The resulting plot is presented at figure 7.2.

Again, the difference between three analysis methods is marginal because there are no highly differentiated SNPs in the data set, and one sub-population is presented by cases only. Still, the signals at chromosome one and three slightly improved, while these at two and X went down.

Load and analyse the data set presented in file `stratified.RData`. GWA data presented in this file concern a study containing data from several populations. All these populations originate from the same base population some generations ago. Some of these populations maintained large size and some were small. There was little (2.5%) migration between populations.

Two traits ('`quat`' and '`bint`') are available for analysis. Investigate relations between phenotypes and covariates. Perform association analysis. Answer the questions

**Ex. 1** — What covariates are significantly associated with the traits?

**Ex. 2** — How many SNPs and IDs are presented in the data set?

**Ex. 3** — How many SNPs and IDs pass the quality control (use SNP and ID call rate of 0.98)?

**Ex. 4** — Is there evidence for stratification coming from the distribution of GW test for HWE (what is  $\lambda$ )?

**Ex. 5** — Is there evidence that the test statistics for trait `quat` is inflated (what is  $\lambda$ )?

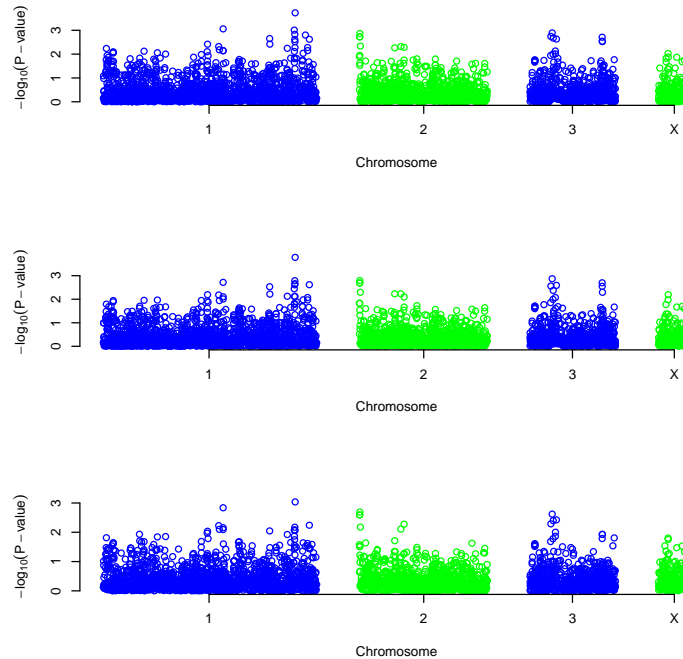


Figure 7.2: Comparison of method of Price et al., principal components adjustment and mixed modelling.

**Ex. 6** — Is there evidence that the test statistics for trait `bint` is inflated (what is  $\lambda$ )?

**Ex. 7** — How many genetically distinct populations are present in the data set? How many people belong to each population?

**Ex. 8** — Is the case/control and quantitative trait disbalance between populations?

**Ex. 9** — For the quantitative trait, what method corrects best for stratification (in terms of minimal residual inflation)?

**Ex. 10** — What is the strongest SNP associated with trait `quat`? What model (method and covariates used) gives best results? Is the finding GW-significant?

**Ex. 11** — What is the strongest SNP associated with trait `bint`? What model (method and covariates used) gives best results? Is the finding GW-significant?

## 7.2 Analysis of family data

In this section we will consider analysis of quantitative traits in a family-based cohort, where participants were not selected for the value of the trait under analysis. Such data may be generated in any study selecting participants based

on kinship (e.g. collections of sibships, nuclear or extended families); also any study in a genetically isolated population is likely to end up with a large proportion of relatively closely related individuals, even if ascertainment was random with the respect to kinship.

In pedigree-based association analysis the pedigree works as a confounder – exactly in the same manner as ethnic origin may work in a population-based study. Any genetic polymorphism is inherited through genealogy, and therefore genotypes are more similar between close relatives. In the same manner, any other heritable trait will be also more similar between relatives, and therefore certain degree of association is expected between *any* genetic marker and *any* heritable trait in a family-based sample. If additive 1 d.f. test for association is considered, the effect of confounding by pedigree can be shown to inflate the resulting null distribution of presumably  $\chi^2_1$  test statistics by a certain constant  $\lambda$ .

As you remember, this is exactly what happens when simple test for association is applied to a population-based data with ethnic admixture. In a population-based study with strong admixture (both in terms of the proportion and ethnic "distance"), some genomic regions may have been differentially selected in different populations. In such situation, use of genomic control does not prevent false-positive association between a trait and these regions, and other methods, such as EIGENSTRAT or Structured Association, are to be used.

For pedigree-based data coming from (relatively) genetically homogeneous population it can be shown that  $\lambda$  is a function of trait's heritability and pedigree structure, expressed as kinship matrix. Thus, genomic control is a simple and valid method to study association in genetically homogeneous families. However, this method reduces (or summarises if you prefer) all the abundant information about heritability and relationship into a single parameter  $\lambda$ , therefore it is not the most powerful method.

In quantitative genetics, a mixed polygenic model of inheritance may be considered as "industrial standard" – this model has sound theoretical bases and is proven by time to describe well inheritance of complex quantitative traits. This model describes the vector of observed quantitative traits as

$$Y = \mu + G + e \quad (7.1)$$

where  $\mu$  is the intercept,  $G$  is contribution from polygene, and  $e$  is random residual.

It is assumed that for each individual its "personal" random residual  $e_i$  is distributed as Normal with mean zero and variance  $\sigma_e^2$ . As these residuals are independent between pedigree members, the joint distribution of residuals in the pedigree can be modelled using multivariate normal distribution with variance-covariance matrix proportional to the identity matrix  $I$  (this is a matrix with diagonal elements equal to 1, and off-diagonal elements equal to zero):  $e \sim MVN(0, I\sigma_e^2)$ .

The polygenic component  $G$  describes the contribution from multiple independently segregating genes all having a small additive effect onto the trait (infinitesimal model). For a person for whom parents are not known, it is assumed that  $G_i$  is distributed as Normal with mean zero and variance  $\sigma_G^2$ . Assuming model of infinitely large number of genes, it can be shown that given polygenic values for parents, the distribution of polygene in offspring follows Normal distribution with mean  $(G_m + G_f)/2$  and variance  $\sigma_G^2/2$ , where  $G_m$  is maternal

and  $G_f$  is paternal polygenic values. From this, it can be shown that jointly the distribution of polygenic component in a pedigree can be described as multivariate normal with variance-covariance matrix proportional to the relationship matrix  $\Phi$ :  $G \sim MVN(0, \Phi\sigma_G^2)$ .

Thus the log-likelihood for this model can be written as a function of three parameters:

$$L(\mu, \sigma_G^2, \sigma_e^2) = -\frac{1}{2} \cdot \log_e |(\Phi \cdot \sigma_G^2 + I \cdot \sigma_e^2)| + (Y - \mu)^T \cdot (\Phi \cdot \sigma_G^2 + I \cdot \sigma_e^2)^{-1} \cdot (Y - \mu) \quad (7.2)$$

where  $\mu$  is intercept,  $\sigma_G^2$  is the proportion of variance explained by the polygenic component, and  $\sigma_e^2$  is the residual variance.

Covariates such as sex, age, or a genetic marker studied for association can be easily included into the model:

$$Y = \mu + \sum_j \beta_j \cdot C_j + G + e$$

Here,  $C_j$  is the vector containing  $j$ -th covariate and  $\beta_j$  is the coefficient of regression of  $Y$  onto that covariate.

This mixed model leads to likelihood

$$L(\mu, \sigma_G^2, \sigma_e^2, \beta_1, \beta_2, \dots) = -\frac{1}{2} \cdot \log_e |(\Phi \cdot \sigma_G^2 + I \cdot \sigma_e^2)| + \left( Y - (\mu + \sum_j \beta_j \cdot C_j) \right)^T \cdot (\Phi \cdot \sigma_G^2 + I \cdot \sigma_e^2)^{-1} \cdot \left( Y - (\mu + \sum_j \beta_j \cdot C_j) \right) \quad (7.3)$$

This general formulation can be easily adopted to test genetic association; for example, an effect of a SNP can be incorporated into regression model

$$Y = \mu + \beta_g \cdot g + G + e$$

where  $g$  is the vector containing genotypic values. In this model, you can specify a variety of 1 d.f. models by different coding of the vector  $g$ . For example, if you consider an "AG" polymorphism and want to estimate and test additive effect of the allele "G", you should code "AA" as 0 (zero), "AG" as 1 and "GG" as 2. Under this coding, the  $\beta_g$  will estimate additive contribution from the "G" allele. If you are willing to consider dominant model for G, you should code "AA" and "AG" as 0 and "GG" as 1. Recessive and over-dominant models can be specified in a similar manner. If, however, you want to estimate general 2 d.f. model, the specification should be different:

$$Y = \mu + \beta_a \cdot g + \beta_d \cdot I_{g=2} + G + e$$

where  $g$  is coded as 0, 1 or 2, exactly the same as in the additive model, and  $I_{g=2}$  is the binary indicator which takes value of one when  $g$  is equal to 2 and zero otherwise. In this model,  $\beta_a$  will estimate the additive and  $\beta_d$  – the dominance



effect. There may be other, alternative coding(s) allowing for essentially the same model, for example

$$Y = \mu + \beta_1 \cdot I_{g=1} + \beta_2 \cdot I_{g=2} + G + e$$

would estimate trait's deviation in these with  $g = 1$  ( $\beta_1$ ) and these with  $g = 2$  ( $\beta_2$ ) from the reference ( $g = 0$ ).

The classical way to estimate mixed polygenic model and test for significance is Maximum Likelihood (ML) or Restricted ML (REML) using equation (7.3). However, when large pedigrees are analysed, ML/REML solution may take prohibitively long time, i.e. from minutes to hours for single SNP analysis, making study of hundreds of thousand of SNPs impossible. Therefore fast approximate tests were developed for the purposes of GWA association analysis in samples of relatives.

Here we will cover two of fast approximations available, FAmily-based Score Test for Association (FASTA, Chen & Abecasis, 2007) and Genome-wide Rapid Analysis using Mixed Models And Score test (GRAMMAS, Amin et al, 2007). Both tests are based on the classical polygenic mixed model and are performed in two steps.

First, polygenic model as specified by equation (7.1) and likelihood (7.2) is estimated using available data.

Secondly, the maximum likelihood estimates (MLEs) of the intercept,  $\hat{\mu}$ , proportion of variance explained by the polygenic component,  $\hat{\sigma}_G^2$ , and residual variance,  $\hat{\sigma}_e^2$ , are used to compute the FASTA test statistics

$$T_F^2 = \frac{((g - E[g])^T \cdot (\Phi \cdot \hat{\sigma}_G^2 + I \cdot \hat{\sigma}_e^2)^{-1} \cdot (Y - \hat{\mu}))^2}{(g - E[g])^T \cdot (\Phi \cdot \hat{\sigma}_G^2 + I \cdot \hat{\sigma}_e^2)^{-1} \cdot (g - E[g])}$$

It can be shown that  $T_F^2$  follows  $\chi_1^2$  when pedigree structure is 100% complete and 100% correct. As this is never actually the case, application of GC to correct for residual inflation is recommended.

FASTA test results in unbiased estimates of the SNP effect and correct  $P$  - values. Please keep in mind that this is correct – as for any score test – only when alternative is reasonably close to the null, i.e. when the SNP explains small proportion of trait's variance. Disadvantages of this test are that it can be relatively slow when thousands of study subjects are analysed, and that permutation procedures can not be applied to estimate genome-wide significance, because the data structure is not exchangeable.

Other test, GRAMMAS, also exploits MLEs from the polygenic model (7.1). However, these are used to first compute the vector of environmental residuals  $\hat{e}$ , using standard equation

$$\hat{e} = \hat{\sigma}_e^2 \cdot (\Phi \cdot \hat{\sigma}_G^2 + I \cdot \hat{\sigma}_e^2)^{-1} \cdot (Y - \hat{\mu})$$

These residuals, in turn, are used to run simple score test:

$$T_G^2 = \frac{((g - E[g])^T \cdot \hat{e})^2}{(g - E[g])^T \cdot (g - E[g])}$$

This test is conservative, but GC can be used to correct for the deflation of the test statistics.

The fact that environmental residuals  $\hat{e}$  are not dependent on pedigree structure leads to a nice property of the GRAMMAS test: the data structure becomes exchangeable and permutations may be used to estimate genome-wide significance. When used in combination with GC,  $P$  – values derived from GRAMMAS test are correct; however, there is a downward bias in estimates of SNP effects.

When using FASTA or GRAMMAS test, it is recommended to estimate genomic kinship matrix from available genome-wide data, and use it in analysis instead of pedigree kinship. This solution firstly does not rely on the completeness and quality of pedigree; secondly, genomic kinship is more likely to give a better estimate of a *true* covariance between individual genomes, while pedigree kinship provides one with expectation. Therefore use of genomic kinship is expected to lead to better estimates of polygenic model, and thus better power to detect association in GWA analysis. This being said, we generally advocate use of genomic, and not pedigree kinship. Of cause, you can only implement this solution when you have GWA data; in a candidate gene study you will have to rely on the pedigree structure to estimate kinship matrix.

### 7.3 Example GWA analysis using family-based data

In this section, we will explore small data set (150 people, 5827 SNPs). Let us load and explore it:

```
> load("RData/erfsmall.RData")
> ls()

[1] "erfs"  "pkins"

> class(erfs)

[1] "gwaa.data"
attr(,"package")
[1] "GenABEL"

> class(pkins)

[1] "matrix"
```

You can see that there are two objects, **erfs** and **pkins**, presented in the data. The class of the first object is standard **GenABEL**-package's **gwaa.data**-class; this is the object containing GWA data. The other object contains kinship matrix, as estimated from pedigree data.

You can check the number of people and SNPs in the data set with

```
> nids(erfs)

[1] 150

> nsnps(erfs)

[1] 5827
```

As usual, it is advisable to check the distribution of SNPs by chromosome:

```
> table(chromosome(erfs))
```

```
 1 10 11 12 13 14 15 16 17 18 19  2 20 21 22 23  3  4  5  6
484 251 224 285 188 210 206 189 159 170 149 481 130 100 132 26 403 300 320 397
 7  8  9  X
286 253 206 278
```

(here, 23 stays for pseudo-autosomal region of the X chromosome); you can see that markers are evenly spread over the chromosomes.

Summary marker statistics can be generated by

```
> descriptives.marker(gtdata(erfs))
```

```
$`Minor allele frequency distribution`
      X<=0.01 0.01<X<=0.05 0.05<X<=0.1 0.1<X<=0.2    X>0.2
No      17.000      26.000      75.000      437.000 5272.000
Prop     0.003      0.004      0.013      0.075   0.905

$`Cumulative distr. of number of SNPs out of HWE, at different alpha`
      X<=1e-04 X<=0.001 X<=0.01 X<=0.05 all X
No           2    14.000   83.000 319.000 5827
Prop          0     0.002    0.014   0.055    1

$`Distribution of proportion of successful genotypes (per person)`
      X<=0.9 0.9<X<=0.95 0.95<X<=0.98 0.98<X<=0.99 X>0.99
No          0      1.000      2.000      12.00  135.0
Prop         0      0.007      0.013      0.08   0.9

$`Distribution of proportion of successful genotypes (per SNP)`
      X<=0.9 0.9<X<=0.95 0.95<X<=0.98 0.98<X<=0.99 X>0.99
No      77.000     33.000     214.000     208.000 5295.000
Prop    0.013      0.006      0.037      0.036   0.909

$`Mean heterozygosity for a SNP`
[1] 0.4402752

$`Standard deviation of the mean heterozygosity for a SNP`
[1] 0.08287253

$`Mean heterozygosity for a person`
[1] 0.4354001

$`Standard deviation of mean heterozygosity for a person`
[1] 0.01305448
```

You can see that the quality of genotypic data is quite reasonable: call rate is generally high, both per-person and per SNP, and there is little deviation from Hardy-Weinberg equilibrium.

Let us explore pedigree kinship matrix. First, let us just look how this matrix looks like by displaying few elements from the upper-left corner:

```
> pkins[1:5,1:5]
```

	id1	id2	id3	id4	id5
id1	5.00000e-01	8.56146e-05	1.01984e-04	2.33397e-04	8.56146e-05
id2	8.56146e-05	5.00000e-01	3.96513e-03	2.56896e-05	2.51269e-01
id3	1.01984e-04	3.96513e-03	5.00000e-01	1.21593e-05	3.96513e-03
id4	2.33397e-04	2.56896e-05	1.21593e-05	5.00000e-01	2.56896e-05
id5	8.56146e-05	2.51269e-01	3.96513e-03	2.56896e-05	5.00000e-01

By definition, pedigree kinship should take values between 0 and 0.5 (plus some small amount from inbreeding); kinship between (non-inbred) sibs or an offspring and the parent is 1/4. You can see that in the upper-left corner there is one inbred sib-pair (or parent-offspring pair; "id2" and "id5"). You can also see that this matrix is symmetric around the diagonal.

Let us summarise the distribution of kinship coefficients; in doing this we want to generate the summary for every off-diagonal element only once. Function `lower.tri` can be used to get the "lower triangle" sub-matrix elements:

```
> summary(pkins[lower.tri(pkins)])
```

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
0.0000000	0.0004999	0.0028500	0.0062810	0.0053780	0.2633000

As you can see, average relationship corresponds to that expected between second cousins ( $1/64 = 0.015625$ ) and third cousins ( $1/256 = 0.00390625$ ).

We can also draw a histogram of the distribution of the kinship coefficients (shown at figure 7.4A):

```
> hist(pkins[lower.tri(pkins)])
```

and see that most elations are indeed remote.

Let us estimate genomic kinship matrix using autosomal data with the command `ibs`, and look up the elements in the upper-left corner:

```
> gkins <- ibs(erfs[,autosomal(erfs)],weight="freq")
> gkins[1:5,1:5]
```

	id1	id2	id3	id4	id5
id1	0.513083834	5.439000e+03	5.446000e+03	5441.00000000	5440.00000000
id2	-0.012569446	4.931959e-01	5.524000e+03	5524.00000000	5521.00000000
id3	0.001516184	-8.551323e-03	5.044065e-01	5529.00000000	5528.00000000
id4	0.010459422	-1.471218e-02	-3.467894e-03	0.50739823	5523.00000000
id5	-0.007957596	2.561484e-01	-8.925127e-03	-0.02205665	0.4943105

Here, the estimated kinship is shown below the diagonal, and the number of informative SNP pairs used for estimation is shown above the diagonal.

You can see that "genomic kinship" coefficients may take values lower than zero, which is consequence of the fact that in effect "genomic kinship" is simply covariance between the vectors of individual genotypes. This quantity, though it provides an unbiased estimate of kinship, can be lower than zero.

```
> summary(gkins[lower.tri(gkins)])
```

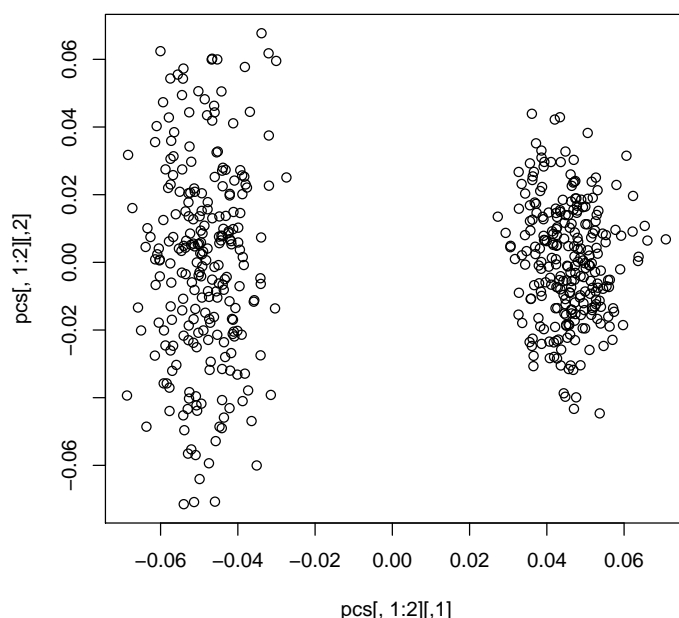


Figure 7.3: Population structure of 'strdat1' data

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
-0.038980	-0.011930	-0.005826	-0.003362	0.000481	0.268000

here, the average is quite close to that obtained with pedigree kinship.

We can also draw a histogram of the distribution of "genomic kinship" coefficients (shown at figure 7.4B):

```
> hist(gkins[lower.tri(gkins)])
```

and can easily graphically present relations between genomic and pedigree kinship with

```
> plot(pkins[lower.tri(pkins)],gkins[lower.tri(gkins)])
```

(shown at figure 7.5), and estimate correlation between the two with

```
> cor(pkins[lower.tri(pkins)],gkins[lower.tri(gkins)])
```

```
[1] 0.9161499
```

From the graph, you can clearly see that, though there is a very strong correlation between genomic and pedigree kinships, these are not identical.

In real data, you may find that there are some points where pedigree data clearly suggest relation different from that suggested by genomic data. Which one to believe? Generally, pedigrees are more prone to errors than genotypic

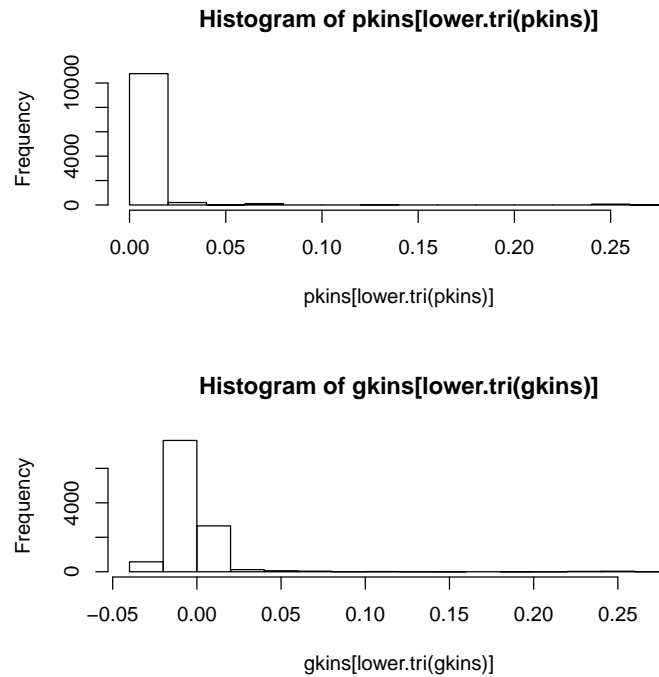


Figure 7.4: Distribution of the pedigree (upper histogram) and genomic (lower histogram) kinship coefficients for `erfs` data set.

data. In the data containing close relatives it is better to rely on "genomic kinship".

Let us first analyse the data using plain GC method:

```
> qts <- qtscore(qtbas, data=erfs)
```

You can check the estimate of the inflation factor  $\lambda$  with

```
> lambda(qts)$est
```

```
[1] 1.125012
```

This is relatively high value, suggesting presence of close relatives in data and high heritability of the trait.

The top 10 hits from GWA analysis can be displayed with

```
> descriptives.scan(qts, sort="Pc1df")
```

Summary for top 10 results, sorted by Pc1df

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB	chi2.1df
rs1781670	13	2694164735	u	1	2	150	0.5236543	0.1348423	15.08125
rs1872087	17	3329938025	u	1	2	150	-0.4958918	0.1329806	13.90584
rs1043883	8	1745120471	u	1	2	128	0.5132294	0.1403802	13.36630
rs377859	10	2173644540	u	1	2	150	-0.5901680	0.1625124	13.18797

```

rs1861659      16 3171591122      u 1 2 150 0.5270266 0.1478622 12.70430
rs1672299      12 2602365489      u 1 2 150 -0.5149239 0.1469410 12.28006
rs11062        17 3262519467      u 1 2 150 0.5321081 0.1580270 11.33801
rs2141693      12 2590653716      u 1 2 150 1.3143719 0.3986408 10.87109
rs716623       13 2749359437      u 1 2 150 0.4724783 0.1458132 10.49953
rs774033       12 2531521679      u 1 2 150 -0.4601787 0.1435537 10.27601
               P1df      effAB      effBB  chi2.2df      P2df      Pc1df
rs1781670 0.0001029814 0.6906708 1.0317669 15.84361 0.0003627468 0.0002508996
rs1872087 0.0001921999 -0.5500476 -0.9839850 13.98868 0.0009170561 0.0004384850
rs1043883 0.0002561863 0.3204631 1.1004272 14.19845 0.0008257431 0.0005670857
rs377859 0.0002817517 -0.5794118 -1.1877540 13.19253 0.0013654589 0.0006174836
rs1861659 0.0003648165 0.3450954 1.1140306 13.79358 0.0010110248 0.0007781712
rs1672299 0.0004578242 -0.6530651 -0.9961549 12.87154 0.0016031702 0.0009536341
rs11062 0.0007593646 0.4922344 1.1305957 11.42110 0.0033108545 0.0015003962
rs2141693 0.0009767716 1.3143719 NA 10.87109 0.0009767716 0.0018800745
rs716623 0.0011940492 0.1922801 0.9718987 12.66176 0.0017804639 0.0022508609
rs774033 0.0013477134 -0.5442070 -0.9178935 10.45943 0.0053550526 0.0025088454

```

here, nominal  $P$  – values after genomic control are given in column named "Pc1df".

We can estimate genome-wide empirical significance by using the same function with `times` argument, which tells the number of permutations:

```

> qts.e <- qtscore(qtbas,data=erfs,times=200,quiet=TRUE)

|
|
|
|=====| 100%

> descriptives.scan(qts.e,sort="Pc1df")

```

Summary for top 10 results, sorted by Pc1df

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB
rs1781670	13	2694164735	u	1	2 150	0.52365430	0.1348423	
rs1872087	17	3329938025	u	1	2 150	-0.49589178	0.1329806	
rs1043883	8	1745120471	u	1	2 128	0.51322939	0.1403802	
rs377859	10	2173644540	u	1	2 150	-0.59016796	0.1625124	
rs1861659	16	3171591122	u	1	2 150	0.52702662	0.1478622	
rs1672299	12	2602365489	u	1	2 150	-0.51492394	0.1469410	
rs11062	17	3262519467	u	1	2 150	0.53210805	0.1580270	
rs2017143	1	2252811	u	1	2 150	0.04200869	0.1338613	
rs2840531	1	2318570	u	1	2 150	-0.35176535	0.2002343	
rs2477703	1	2458154	u	1	2 150	-0.14676181	0.1519094	
	chi2.1df	P1df	Pc1df	effAB	effBB	chi2.2df	P2df	
rs1781670	15.08124536	0.325	0.660	0.6906708	1.03176686	15.8436112	0.730	
rs1872087	13.90584450	0.545	0.840	-0.5500476	-0.98398504	13.9886838	0.970	
rs1043883	13.36630002	0.665	0.895	0.3204631	1.10042723	14.1984536	0.970	
rs377859	13.18797157	0.685	0.910	-0.5794118	-1.18775397	13.1925294	0.990	
rs1861659	12.70429594	0.800	0.955	0.3450954	1.11403056	13.7935817	0.980	
rs1672299	12.28005767	0.845	0.970	-0.6530651	-0.99615492	12.8715445	0.995	

```
rs11062    11.33801475 0.950 0.995  0.4922344  1.13059573 11.4210979 1.000
rs2017143  0.09848463 1.000 1.000  0.2555726  0.01458913  1.5429109 1.000
rs2840531  3.08623677 1.000 1.000 -0.3889020 -0.36730336  3.2760351 1.000
rs2477703  0.93337596 1.000 1.000 -0.1681636 -0.27086570  0.9548231 1.000
```

(argument "quiet" suppress warning messages; this is used for the purposes of this tutorial, normally you do not need to specify this option)

As you can see, in this analysis nothing comes even close to genome-wide significance, as indicated by genome-wide corrected  $P$ -values (column "Pc1df") all  $>> 0.05$ .

Let us estimate polygenic model with

```
> h2 <- polygenic(qtbas, kin=gkins, data=erfs)
```

The results of estimation are contained in "h2an" element of the resulting analysis object:

```
> h2$h2an

$minimum
[1] 200.9972

$estimate
[1] 0.03850405 0.77968841 1.54153952

$gradient
[1] -1.421085e-07 -5.684342e-08  1.651780e-07

$code
[1] 1

$iterations
[1] 12
```

In the "estimate" list, the MLEs shown correspond to intercept  $\hat{\mu}$ , heritability  $\hat{h}^2 = \hat{\sigma}_G^2 / (\hat{\sigma}_G^2 + \hat{\sigma}_e^2)$ , and total variance  $\hat{\sigma}_T^2 = \hat{\sigma}_G^2 + \hat{\sigma}_e^2$ . You can see that heritability of the trait is indeed high – almost 80%.

Under these conditions (high heritability, presence of close relatives) we may expect that FASTA and GRAMMAS analysis exploiting heritability model and relationship matrix in exact manner may have better power compared to simple GC.

Let us run FASTA test using estimated polygenic model, as specified by h2 object:

```
> mms <- mmscore(h2, data=erfs)
```

There is little residual inflation left when we use "genomic kinship" matrix:

```
> lambda(mms)$est

[1] 1
```

And the significance of "top" hit becomes an order of magnitude better compared to plain GC:



```
> descriptives.scan(mms,sort="Pc1df")
```

Summary for top 10 results, sorted by Pc1df

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB	chi2.1df
rs1781670	13	2694164735	u	1	2	150	0.5280481	0.1444019	13.372139
rs1953817	6	1403824983	u	1	2	150	-0.5141566	0.1543596	11.094902
rs11062	17	3262519467	u	1	2	150	0.5409064	0.1624135	11.091753
rs774033	12	2531521679	u	1	2	150	-0.4852698	0.1457531	11.084874
rs1043883	8	1745120471	u	1	2	128	0.4873708	0.1515702	10.339302
rs1672299	12	2602365489	u	1	2	150	-0.4829157	0.1519733	10.097360
rs2384933	16	3139192007	u	1	2	150	-0.5466347	0.1723503	10.059355
rs1370139	6	1381958118	u	1	2	149	0.6704706	0.2116017	10.039709
rs1054889	2	324154337	u	1	2	150	0.4765958	0.1509593	9.967379
rs896986	2	336951524	u	1	2	150	-0.4735895	0.1513640	9.789461

	P1df	Pc1df	effAB	effBB	chi2.2df	P2df
rs1781670	0.0002553899	0.0002553899	NA	NA	0	NA
rs1953817	0.0008656536	0.0008656536	NA	NA	0	NA
rs11062	0.0008671249	0.0008671249	NA	NA	0	NA
rs774033	0.0008703475	0.0008703475	NA	NA	0	NA
rs1043883	0.0013022725	0.0013022725	NA	NA	0	NA
rs1672299	0.0014848188	0.0014848188	NA	NA	0	NA
rs2384933	0.0015157632	0.0015157632	NA	NA	0	NA
rs1370139	0.0015320140	0.0015320140	NA	NA	0	NA
rs1054889	0.0015933812	0.0015933812	NA	NA	0	NA
rs896986	0.0017551493	0.0017551493	NA	NA	0	NA

If you compare these results to that obtained with simple GC, you can also see that the ranks of top hits have changed quite a bit; unbiased estimated of genetic effects were obtained.

However, we can not estimate genome-wide significance with FASTA, because the data structure is not exchangeable.

Using GRAMMAS method, you can estimate nominal  $P$  – values by

```
> grs <- qtscore(h2$pgres,data=erfs,clam=FALSE)
> lambda(grs)$est
```

```
[1] 0.7864778
```

In the above analysis, note that the estimated "inflation" factor  $\lambda$  is less than one, i.e. now it is the GRAMMAS *deflation* factor. In order to obtain non-conservative test statistics, we had to say to qtscore that deflation is OK (parameter clam=FALSE).

We can see "top" nominal corrected  $P$  – values with

```
> descriptives.scan(grs,sort="Pc1df")
```

Summary for top 10 results, sorted by Pc1df

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB
rs1781670	13	2694164735	u	1	2	150	0.10543625	0.03316109
rs1953817	6	1403824983	u	1	2	150	-0.11570443	0.03763454
rs774033	12	2531521679	u	1	2	150	-0.10777281	0.03530344
rs11062	17	3262519467	u	1	2	150	0.11723795	0.03886279

```

rs988457      X 4318354390      u  1  2 150  0.08596545 0.02973400
rs1672299     12 2602365489      u  1  2 150 -0.10338769 0.03613645
rs1054889      2  324154337      u  1  2 150  0.10060789 0.03564684
rs1043883      8 1745120471      u  1  2 128  0.09719095 0.03495001
rs2384933     16 3139192007      u  1  2 150 -0.10913661 0.03958013
rs7141672     14 2851979738      u  1  2 150  0.10188182 0.03716035
      chi2.1df      P1df      effAB      effBB      chi2.2df      P2df
rs1781670 10.109331 0.001475205  0.10640049  0.2107828 10.109751 0.006378161
rs1953817  9.452063 0.002109117 -0.12909511 -0.2188059  9.586110 0.008287102
rs774033   9.319322 0.002267499 -0.12787712 -0.2149561  9.492933 0.008682320
rs11062    9.100568 0.002555302  0.08864787  0.2820712  9.806829 0.007421199
rs988457    8.358747 0.003838352  0.01088472  0.1705728  8.902349 0.011664860
rs1672299    8.185525 0.004222595 -0.12352309 -0.2018643  8.393310 0.015045819
rs1054889    7.965666 0.004767296  0.05921009  0.2144888  8.894878 0.011708513
rs1043883    7.733159 0.005421582  0.04374657  0.2148896  8.765118 0.012493348
rs2384933    7.603028 0.005827035 -0.11565587 -0.2057438  7.643917 0.021884901
rs7141672    7.516813 0.006112573  0.07576841  0.2356800  8.024857 0.018089412
      Pc1df
rs1781670 0.0003367712
rs1953817 0.0005268300
rs774033  0.0005767867
rs11062   0.0006697772
rs988457  0.0011138349
rs1672299 0.0012548232
rs1054889 0.0014601180
rs1043883 0.0017144147
rs2384933 0.0018758833
rs7141672 0.0019912707

```

By comparing this output to that from FASTA test, you can see that  $P$ -values are quite close, but the effects are underestimated with GRAMMAS, as expected.

However, the strengths of GRAMMAS test is not only its speed, but also possibility to estimate genome-wide significance. This can be done by

```

> grs.e <- qtscore(h2$pgres,data=erfs,times=200,clam=FALSE,quiet=TRUE)

|
|
|
|=====| 100%

> descriptives.scan(grs.e,sort="Pc1df")

Summary for top 10 results, sorted by Pc1df
      Chromosome  Position Strand A1 A2  N      effB      se_effB
rs1781670       13 2694164735      u  1  2 150  0.10543625 0.03316109
rs1953817        6 1403824983      u  1  2 150 -0.11570443 0.03763454
rs774033        12 2531521679      u  1  2 150 -0.10777281 0.03530344
rs11062         17 3262519467      u  1  2 150  0.11723795 0.03886279
rs988457        X 4318354390      u  1  2 150  0.08596545 0.02973400

```

rs1672299	12	2602365489	u	1	2	150	-0.10338769	0.03613645
rs1054889	2	324154337	u	1	2	150	0.10060789	0.03564684
rs1043883	8	1745120471	u	1	2	128	0.09719095	0.03495001
rs2017143	1	2252811	u	1	2	150	0.02121630	0.03291983
rs2840531	1	2318570	u	1	2	150	-0.06596746	0.04924261
	chi2.1df	P1df	Pc1df		effAB		effBB	chi2.2df P2df
rs1781670	10.1093309	0.995	0.715		0.10640049		0.21078277	10.1097511 1
rs1953817	9.4520634	1.000	0.850		-0.12909511		-0.21880586	9.5861099 1
rs774033	9.3193222	1.000	0.880		-0.12787712		-0.21495609	9.4929330 1
rs11062	9.1005678	1.000	0.910		0.08864787		0.28207116	9.8068293 1
rs988457	8.3587468	1.000	0.975		0.01088472		0.17057284	8.9023487 1
rs1672299	8.1855251	1.000	0.985		-0.12352309		-0.20186430	8.3933102 1
rs1054889	7.9656656	1.000	0.995		0.05921009		0.21448884	8.8948782 1
rs1043883	7.7331587	1.000	0.995		0.04374657		0.21488962	8.7651179 1
rs2017143	0.4153596	1.000	1.000		0.04950544		0.03323599	0.8344174 1
rs2840531	1.7946401	1.000	1.000		-0.07118473		-0.08469889	1.8565797 1

As you can see, now the "top" hit starts approaching genome-wide significance (genome-wide  $P$ -value  $\sim 10\%$ ), showing the power of kinship-based methods under high heritability model.

Finally, let us plot  $-\log_{10}$  nominal  $P$ -values from different methods across the genome. Let black dots correspond to GC, green to GRAMMAS and red to FASTA (figure 7.6):

```
> plot(mms,df="Pc1df")
> add.plot(grs,df="Pc1df",col=c("lightgreen","lightblue"),cex=1.2)
> add.plot(qts)
```

You can see that there is a great degree of correlation between the FASTA and GRAMMAS  $P$ -values, while plain GC really stands apart.

## 7.4 Exercise: analysis of family data

**Exercise 1** Repeat heritability estimation, FASTA and GRAMMAS analysis of previous section using pedigree kinship (*pkins* object). Discuss the results.

In the next section, you will explore a small (695 people) subset of people from ERF, a family-based study with participants coming from a genetically isolated population and sampled based on kinship (all living descendants of 22 couples living in the area in mid-XIX<sup>th</sup> century). The study participants were genotyped using Illumina 6K "linkage" array. QC was already performed. Your trait of interest is "qtbas".

Explore the data set and answer the questions:

**Exercise 2** Describe the trait "qt". Can you detect significant outliers at visual inspection? Is trait distributed normally? What are significant covariates?

**Exercise 3** Explore relations between genomic and pedigree kinship (these are provided in data as *gkin* and *pkln* data objects, respectively). What are your conclusions? Which matrix would you use later on?

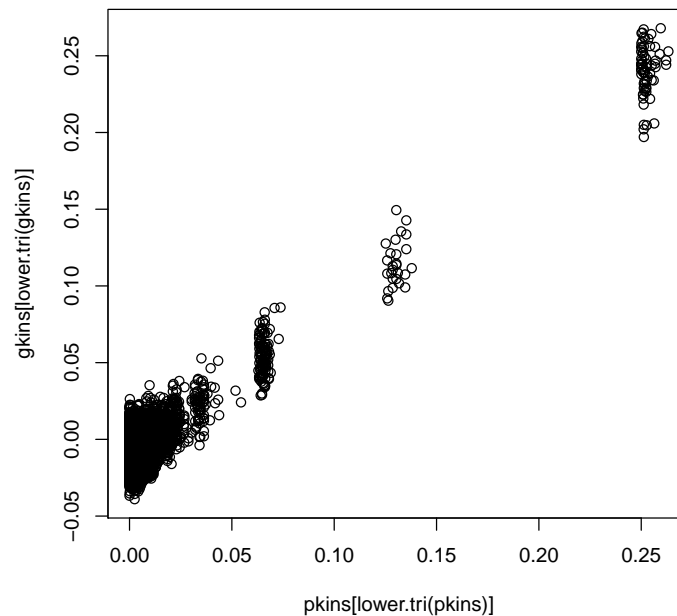


Figure 7.5: Scatter-plot relating pedigree and genomic kinships for `erfs` data set.

**Exercise 4** *What is the heritability of the trait (take care: polygenic analysis may rung for a long while)? Based on heritability analysis, how would you rank different methods of GWA analysis for this trait (and why)?*

**Exercise 5** *Do GWA analysis using simple score test with genomic control. Estimate genome-wide significance. What are your conclusions?*

*Run GWA analysis using the "best" method and model as you have decided in previous exercises. Estimate genome-wide significance. What are your conclusions? Did they change compared to simple analysis?*

**Exercise 6** *Repeat the last "best" analysis using pedigree kinship. How your results change?*

**Exercise 7** *If you have any time left – repeat analysis using "qt" trait. This one is much more fun, but also more laborous to analyse.*

## 7.5 Answers to exercises

**Answer (Ex. 1)** — Basically, all covariates are significantly associated with the traits:

```
> summary(lm(quat~sex+age+age2,data=phdata(strdat)))
```

Call:

```
lm(formula = quat ~ sex + age + age2, data = phdata(strdat))
```

Residuals:

Min	1Q	Median	3Q	Max
-5.1188	-1.0260	-0.0389	1.0221	5.5506

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.3312562	1.4595518	0.227	0.821
sex	0.9190870	0.1399336	6.568	1.29e-10 ***
age	0.1465840	0.0588066	2.493	0.013 *
age2	-0.0024764	0.0005839	-4.241	2.65e-05 ***

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.559 on 496 degrees of freedom

Multiple R-squared: 0.33, Adjusted R-squared: 0.326

F-statistic: 81.44 on 3 and 496 DF, p-value: < 2.2e-16

```
> summary(glm(bint~sex+age+age2,data=phdata(strdat),family=binomial))
```

Call:

```
glm(formula = bint ~ sex + age + age2, family = binomial, data = phdata(strdat))
```

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.4500	-1.0139	-0.5967	1.1013	2.2229

Coefficients:

	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	-6.280565	2.361455	-2.660	0.007823 **
sex	0.809689	0.198527	4.078	4.53e-05 ***
age	0.305322	0.099277	3.075	0.002102 **
age2	-0.003825	0.001033	-3.702	0.000214 ***

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 673.01 on 499 degrees of freedom

Residual deviance: 605.06 on 496 degrees of freedom

AIC: 613.06

Number of Fisher Scoring iterations: 4

**Answer (Ex. 2)** — How many SNPs and IDs are presented in the data set?

```
> nsnpns(strdat)
```

```
[1] 10000
```

```
> nids(strdat)
[1] 500
```

**Answer (Ex. 3)** — Let us perform the QC:

```
> qc <- check.marker(strdat, call=0.98, perid.call=0.98, p.level=0)
```

Excluding people/markers with extremely low call rate...

10000 markers and 500 people in total

0 people excluded because of call rate < 0.1

0 markers excluded because of call rate < 0.1

Passed: 10000 markers and 500 people

RUN 1

10000 markers and 500 people in total

30 (0.3%) markers excluded as having low (<0.5%) minor allele frequency

0 (0%) markers excluded because of low (<98%) call rate

0 (0%) markers excluded because they are out of HWE (P < 0)

0 (0%) people excluded because of low (<98%) call rate

Mean autosomal HET is 0.2035464 (s.e. 0.004215707)

0 people excluded because too high autosomal heterozygosity (FDR < 1%)

Mean IBS is 0.8290788 (s.e. 0.005669894), as based on 2000 autosomal markers

0 (0%) people excluded because of too high IBS (>=0.95)

In total, 9970 (99.7%) markers passed all criteria

In total, 500 (100%) people passed all criteria

RUN 2

9970 markers and 500 people in total

0 (0%) markers excluded as having low (<0.5%) minor allele frequency

0 (0%) markers excluded because of low (<98%) call rate

0 (0%) markers excluded because they are out of HWE (P < 0)

0 (0%) people excluded because of low (<98%) call rate

Mean autosomal HET is 0.2035464 (s.e. 0.004215707)

0 people excluded because too high autosomal heterozygosity (FDR < 1%)

Mean IBS is 0.8253829 (s.e. 0.005766908), as based on 2000 autosomal markers

0 (0%) people excluded because of too high IBS (>=0.95)

In total, 9970 (100%) markers passed all criteria

In total, 500 (100%) people passed all criteria

```
> strdat1 <- strdat[qc$idok, qc$snpok]
```

The number of IDs and SNPs passing are

```
> nsnpns(strdat1)
```

```
[1] 9970
```

```
> nids(strdat1)
```

```
[1] 500
```

**Answer (Ex. 4)** — Not really:

```
> descriptives.marker(strdat1)[2]
```

```
$`Cumulative distr. of number of SNPs out of HWE, at different alpha`
      X<=1e-04 X<=0.001 X<=0.01 X<=0.05 all X
No           1     6.000  73.000 378.000 9970
Prop          0     0.001   0.007   0.038    1
```

**Answer (Ex. 5)** — Yes:

```
> qts.q <- qtscore(quat~sex+age+age2,strdat1)
> lambda(qts.q)

$estimate
[1] 1.633427

$se
[1] 0.0003823764

$iz0
[1] 1.557426

$iz2
[1] 1.282158
```

**Answer (Ex. 6)** — Yes:

```
> qts.b <- qtscore(bint~sex+age+age2,strdat1)
> lambda(qts.b)

$estimate
[1] 2.093596

$se
[1] 0.000838563

$iz0
[1] 1.947389

$iz2
[1] 1.448017
```

**Answer (Ex. 7)** — Two genetically distinct populations are present in the data set. These can be visualised with

```
> gkin <- ibs(strdat1,w="freq")
> dst <- as.dist(0.5-gkin)
> pcs <- cmdscale(dst,k=10)
> plot(pcs[,1:2])

(graph presented in figure 7.3)
The populations can be distinguished with

> pop <- 1*(pcs[,1]>0)
```

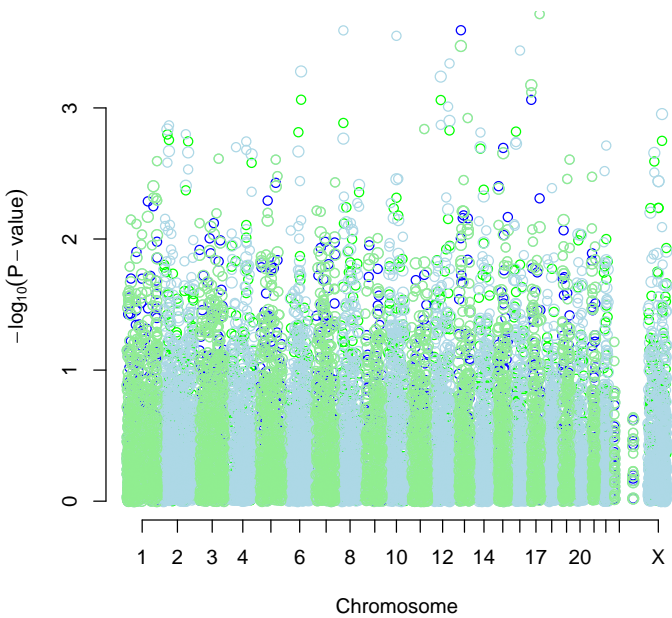


Figure 7.6: Comparison of FASTA (red), GRAMMAS (green), and plain GC (black).

and the number of people belonging to each can be computed with

```
> table(pop)
pop
 0    1
242 258
```

**Answer (Ex. 8)** — Yes, there is significant case/control disbalance:

```
> descriptives.trait(strdat1,by=pop)

      No(by.var=0)      Mean      SD No(by.var=1)      Mean      SD      Ptt      Pkw
id           242         NA       NA           258         NA       NA       NA       NA
sex           242      0.537    0.500           258      0.527    0.500 0.822 0.822
age           242    50.946    9.042           258    49.762    9.897 0.163 0.138
age2          242 2676.962  917.847           258 2573.786  991.693 0.228 0.138
quat          242     2.321    1.751           258     1.119    1.851 0.000 0.000
bint          242     0.657    0.476           258     0.159    0.366 0.000 0.000

      Pexact
id         NA
sex    0.858
age         NA
age2        NA
```



```
quat      NA
bint 0.000
```

**Answer (Ex. 9)** — MMSCORE corrects best for stratification in terms of minimal residual inflation:

```
> sa <- qtscore(quat~sex+age+age2,strdat1,strat=pop)
> lambda(sa)$est
[1] 1.019565

> es <- egsscore(quat~sex+age+age2,strdat1,kin=gkin)
> lambda(es)$est
[1] 1.043936

> pcs <- cmdscale(as.dist(0.5-gkin),k=10)
> pc <- qtscore(quat~sex+age+age2+pcs[,1]+pcs[,2]+pcs[,3],strdat1,strat=pop)
> lambda(pc)$est
[1] 1.025369

> h2an <- polygenic(quat~sex+age+age2,data=strdat1,kin=gkin)
> h2an$h2an
$minimum
[1] 932.8796

$estimate
[1] 1.705618046 0.002208056 0.111974053 -0.002151578 0.802834086
[6] 2.188456701

$gradient
[1] 0.4789398 -211.1951663 11.5282812 29345.2801618 13.2616029
[6] -88.1323148

$code
[1] 4

$iterations
[1] 100

> mm <- mmscore(h2an,strdat1)
> lambda(mm)$est
[1] 1.115282
```

**Answer (Ex. 10)** — The best results are achieved with `mmscore`:

```
> summary(mm)

Summary for top 10 results, sorted by Pldf
```

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB	chi2.1df
snp13598	1	8326962	+	1	2	500	-0.7531795	0.1581588	22.67830
snp358367	19	263284193	+	1	2	500	1.1021752	0.2387578	21.31014

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snp314412	17	231431537	+	1	2	500	-0.9551964	0.2396511	15.88643
snp162350	9	118783325	+	1	2	500	1.6538365	0.4348730	14.46306
snp155145	8	111959587	+	1	2	500	-1.4398364	0.3905136	13.59421
snp293669	16	216390949	+	1	2	500	2.0797905	0.5647042	13.56429
snp102095	6	74672442	+	1	2	500	0.5992210	0.1683224	12.67331
snp123032	7	89813084	+	1	2	500	0.4761745	0.1341130	12.60639
snp289207	15	211238602	+	1	2	500	-1.0830522	0.3110327	12.12514
snp340440	18	249874969	+	1	2	500	-0.3672634	0.1061263	11.97593
	P1df	Pc1df	effAB	effBB	chi2.2df	P2df			
snp13598	1.915188e-06	6.503017e-06	NA	NA	0	NA			
snp358367	3.906601e-06	1.235654e-05	NA	NA	0	NA			
snp314412	6.725914e-05	1.605451e-04	NA	NA	0	NA			
snp162350	1.429350e-04	3.168479e-04	NA	NA	0	NA			
snp155145	2.268844e-04	4.807141e-04	NA	NA	0	NA			
snp293669	2.305293e-04	4.876771e-04	NA	NA	0	NA			
snp102095	3.709122e-04	7.490879e-04	NA	NA	0	NA			
snp123032	3.844301e-04	7.736873e-04	NA	NA	0	NA			
snp289207	4.974669e-04	9.763913e-04	NA	NA	0	NA			
snp340440	5.389224e-04	1.049564e-03	NA	NA	0	NA			

## Chapter 8

# Imperfect knowledge about genotypes

This chapter is mostly copy-pasted from **ProbABEL-package** manual – user is encouraged to check it directly for the latest version.

### 8.1 Motivation

Many statistical and experimental techniques, such as imputations and high-throughput sequencing, generate data which are informative for genome-wide association analysis and are probabilistic in the nature.

When we work with directly genotyped markers using such techniques as SNP or microsatellite typing, we would normally know the genotype of a particular person at a particular locus with very high degree of confidence, and, in case of biallelic marker, can state whether genotype is  $AA$ ,  $AB$  or  $BB$ .

On the contrary, when dealing with imputed or high-throughput sequencing data, for many of the genomic loci we are quite uncertain about the genotypic status of the person. Instead of dealing with known genotypes we work with a probability distribution that is based on observed information, and we have estimates that true underlying genotype is either  $AA$ ,  $AB$  or  $BB$ . The degree of confidence about the real status is measured with the probability distribution  $\{P(AA), P(AB), P(BB)\}$ .

Several techniques may be applied to analyse such data. The most simplistic approach would be to pick up the genotype with highest probability, i.e.  $\max_g [P(g = AA), P(g = AB), P(g = BB)]$  and then analyse the data as if directly typed markers were used. The disadvantage of this approach is that it does not take into account the probability distribution – i.e. the uncertainty about the true genotypic status. Such analysis is statistically wrong: the estimates of association parameters (regression coefficients, odds or hazard ratios, etc.) are biased, and the bias becomes more pronounced with greater probability distribution uncertainty (entropy).

One of the solutions that generate unbiased estimates of association parameters and takes the probability distribution into account is achieved by performing association analysis by means of regression of the outcome of interest onto estimated genotypic probabilities.

The **ProbABEL-package** package was designed to perform such regression in a fast, memory-efficient and consequently genome-wide feasible manner. Currently, **ProbABEL-package** implements linear, logistic regression, and Cox proportional hazards models. The corresponding analysis programs are called **palinear**, **palogist**, and **pacoxph**.

## 8.2 Input files

**ProbABEL-package** takes three files as input: a file containing SNP information (e.g. the MLINFO file of MACH), a file with genome- or chromosome-wide predictor information (e.g. the MLDOSE or MLPROB file of MACH), and a file containing the phenotype of interest and covariates.

Optionally, the map information can be supplied (e.g. the "legend" files of HapMap).

The dose/probability file may be supplied in filevector format in which case **ProbABEL-package** will operate much faster, and in low-RAM mode (approx.  $\approx 128$  MB). See the R libraries **GenABEL-package** and **DatABEL-package** on how to convert MACH and IMPUTE files to filevector format (functions: `mach2databel()` and `impute2databel()`, respectively).

### 8.2.1 SNP information file

In the simplest scenario, the SNP information file is an MLINFO file generated by MACH. This must be a space or tab-delimited file containing SNP name, coding for allele 1 and 2 (e.g. A, T, G or C), frequency of allele 1, minor allele frequency and two quality metrics ("Quality", the average maximum posterior probability and "Rsq", the proportion of variance decrease after imputations).

Actually, for **ProbABEL-package**, it does not matter what is written in this file – this information is just brought forward to the output. However, **it is critical** that the number of columns is seven and the number of lines in the file is equal to the number of SNPs in the corresponding DOSE file (plus one for the header line).

The example of SNP information file content follows here (also to be found in `ProbABEL/examples/test.mlinfo`)

```
SNP A11 A12 Freq1 MAF Quality Rsq
rs7247199 G A 0.5847 0.4150 0.9299 0.8666
rs8102643 C T 0.5847 0.4150 0.9308 0.8685
rs8102615 T A 0.5006 0.4702 0.9375 0.8932
rs8105536 G A 0.5783 0.4213 0.9353 0.8832
rs2312724 T C 0.9122 0.0877 0.9841 0.9232
```

Note that header line is present in the file. The file describes five SNPs.

### 8.2.2 Genomic predictor file

Again, in the simplest scenario this is an MLDOSE or MLPROB file generated by MACH. Such file starts with two special columns plus, for each of the SNPs under consideration, a column containing the estimated allele 1 dose (MLDOSE). In an MLPROB file, two columns for each SNP correspond to posterior

probability that person has two ( $P_{A_1A_1}$ ) or one ( $P_{A_1A_2}$ ) copies of allele 1. The first “special” column is made of the sequential id, followed by an arrow followed by study ID (the one specified in the MACH input files). The second column contains the method keyword (e.g. “MLDOSE”).

An example of the few first lines of an MLDOSE file for five SNPs described in SNP information file follows here (also to be found in the file `ProbABEL/examples/test.mldose`)

```
1->id636728 MLDOSE 0.974 0.974 0.968 0.971 2
2->id890314 MLDOSE 0.947 0.947 0.113 0.944 1.094
3->id102874 MLDOSE 1.005 1.004 NaN 1.002 2
4->id200949 MLDOSE 1.968 1.969 1.973 1.977 2
5->id336491 MLDOSE 1.007 1.006 1.001 1.004 2
6->id988766 MLDOSE 1.006 1.006 1 1.003 2
7->id21999 MLDOSE 1.968 1.969 1.973 1.977 2
8->id433893 MLDOSE 1.006 1.006 1.001 1.004 2
9->id688932 MLDOSE 1.006 1.006 1.001 1.004 2
10->id394203 MLDOSE 1.967 1.968 1.972 1.976 1.999
11->id995678 MLDOSE 1.014 1.014 1.006 1.009 2
```

**The order of SNPs in the SNP information file and DOSE-file must be the same.** This should be the case if you just used MACH outputs.

Therefore, by all means, the number of columns in the genomic predictor file must be the same as the number of lines in the SNP information file plus one.

The dose/probability file may be supplied in filevector format (`.fvi` and `.fvd` files) in which case `ProbABEL` will operate much faster, and in low-RAM mode (approx. 128 MB). On the command line simply specify the `.fvi` file as argument for the `-dose` option (cf. section 8.3 for more information on the options accepted by `ProbABEL`). See the R libraries `GenABEL` and `DatABEL` on how to convert MACH and IMPUTE files to filevector format (functions: `mach2databel()` and `impute2databel()`, respectively).

### 8.2.3 Phenotypic file

The phenotypic data file contains phenotypic data, but also specifies the analysis model. There is a header line, specifying the variable names. The first column should contain personal study IDs. It is assumed that **both the total number and the order of these IDs are exactly the same as in the genomic predictor (MLDOSE) file described in previous section**. This is not difficult to arrange using e.g. R; an example is given in the `ProbABEL/examples` directory.

**Missing data should be coded with 'NA', 'N' or 'NaN' codes.** Any other coding will be converted to some number which will be used in analysis! E.g. coding missing as `'-999.9'` will result in an analysis which will consider `-999.9` as indeed a true measurements of the trait/covariates.

In the case of linear or logistic regression (programs `palinear` and `palogist`, respectively), the second column specifies the trait under analysis, while the third, fourth, etc. provide information on covariates to be included into analysis. An example few lines of phenotypic information file designed for linear regression analysis follow here (also to be found in `ProbABEL/examples/height.txt`)

```
id height sex age
```

```

id636728 174.429795159687 0 56.5664877162697
id890314 168.176943059097 0 74.8311971509938
id102874 178.612190619767 1 45.2478051768211
id200949 171.770230117638 0 46.7362651142108
id336491 185.941629656499 1 61.2743318817997
id988766 173.159286450017 1 43.9794924518567
id21999 167.478282481124 0 64.842094190157
id433893 168.33178468379 1 49.2526444099125
id688932 171.691587811178 0 50.3954417563357
id394203 173.491495887183 1 71.6498502881161

```

Note again that the order of IDs is the same between the MLDOSE file and the phenotypic data file. The model specified by this file is  $height \sim \mu + \text{sex} + \text{age}$ , where  $\mu$  is the intercept.

Clearly, you can for example include  $\text{sex} \times \text{age}$  interaction terms by specifying another column having a product of sex and age here.

For logistic regression, it is assumed that in the second column cases are coded as “1” and controls as “0”. An couple of example lines of a phenotypic information file designed for logistic regression analysis follow here (also to be found in ProbABEL/examples/logist\_data.txt)

```

id chd sex age othercov
id636728 0 0 56.5664877162697 -0.616649220436139
id890314 0 0 74.8311971509938 0.695315865158652
id102874 1 1 45.2478051768211 -0.919192364890525
id200949 0 0 46.7362651142108 -0.623212536893650
id336491 0 1 61.2743318817997 -0.0835744351009496
id988766 0 1 43.9794924518567 -0.360419162609288
id21999 1 0 64.842094190157 -0.180940346913155
id433893 0 1 49.2526444099125 0.126374731789777
id688932 0 0 50.3954417563357 1.06437576032067
id394203 1 1 71.6498502881161 -1.18226498491599

```

You can see that in the first 10 people, there are three cases, as indicated by “chd” equal to one. The model specified by this file is  $\text{chd} \sim \mu + \text{sex} + \text{age} + \text{othercov}$ .

In case of the Cox proportional hazards model, the composition of the phenotypic input file is a bit different. In the second column and third column, you need to specify the outcome in terms of follow-up time (column two) and event (column three, “1” if an event occurred and zero if censored). Columns starting from four (inclusive) specify covariates to be included into the analysis. An example few lines of a phenotypic information file designed for the Cox proportional hazards model analysis follow here (also to be found in ProbABEL/examples/coxph\_data.txt)

```

id fupt_chd chd sex age othercov
id636728 3.187930645 0 0 56.56648772 -0.61664922
id890314 2.099691952 0 0 74.83119715 0.695315865
id102874 9.133488079 1 1 45.24780518 -0.919192365
id200949 7.525406804 0 0 46.73626511 -0.623212537
id336491 6.798229522 0 1 61.27433188 -0.083574435

```

```
id988766 6.149545358 0 1 43.97949245 -0.360419163
id21999 1.013546103 1 0 64.84209419 -0.180940347
id433893 1.282853098 0 1 49.25264441 0.126374732
id688932 8.340206657 0 0 50.39544176 1.06437576
id394203 3.392345681 1 1 71.64985029 -1.182264985
```

You can see that for the first ten people, the event occurs for three of them, while for the other seven there is no event during the follow-up time, as indicated by the “chd” column. Follow-up time is specified in the preceding column. The covariates included into the model are age (presumably at baseline), sex and “othercov”; thus the model, in terms of **R/survival** is

$$\text{Surv(fuptime\_chd, chd)} \sim \text{sex} + \text{age} + \text{othercov}.$$

### 8.2.4 Optional map file

If you would like map information (e.g. base pair position) to be included in your outputs, you can supply a map file. These follow HapMap “legend” file format. For example, for the five SNPs we considered the map-file may look like (example can be found in **ProbABEL/examples/test.map**)

```
rs position 0 1
rs7247199 204938 A G
rs8102643 207859 C T
rs8102615 211970 A T
rs8105536 212033 A G
rs2312724 217034 C T
```

The order of the SNPs in the map file should follow that in the SNP information file. Only information from the second column – the SNP location – is actually used to generate the output.

## 8.3 Running an analysis

To run linear regression, you should use the program called **palinear**; for logistic analysis use **palogist**, and for the Cox proportional hazards model use **pacoxph** (all are found in the **ProbABEL/bin/** directory after you have compiled the program).

There are in total 11 command line options you can specify to the **ProbABEL-package** analysis functions **palinear** or **palogist**. If you run either program without any argument, you will get a short explanation to command line options:

```
user@server:~$ palogist
```

```
Usage: ../bin/palogist options
```

```
Options:
```

```
--pheno      : phenotype file name
--info       : information (e.g. MLINFO) file name
--dose       : predictor (e.g. MLDOSE/MLPROB) file name
--map        : [optional] map file name
```

```

--nids      : [optional] number of people to analyse
--chrom     : [optional] chromosome (to be passed to output)
--out       : [optional] output file name (default is regression.out.txt)
--skipd     : [optional] how many columns to skip in predictor
              (dose/prob) file (default 2)
--ntraits   : [optional] how many traits are analysed (default 1)
--ngpreds   : [optional] how many predictor columns per marker
              (default 1 = MLDOSE; else use 2 for MLPROB)
--separat   : [optional] character to separate fields (default is space)
--score     : use score test
--no-head   : do not report header line
--allcov    : report estimates for all covariates (large outputs!)
--interaction : which covariate to use for interaction with SNP
              (default is no interaction, 0)
--mmscore   : score test for association between a trait and genetic
              polymorphism, in samples of related individuals
--robust    : report robust (aka sandwich, aka Hubert-White) standard
              errors
--help      : print help

```

### 8.3.1 Basic analysis options

However, for a simple run only three options are mandatory, which specify the necessary files needed to run the regression analysis.

These options are `-dose` (or `-d`), specifying the genomic predictor/MLDOSE file described in sub-section 8.2.2; `-pheno` (or `-p`), specifying the phenotypic data file described in sub-section 8.2.3; and `-info` (or `-i`), specifying the SNP information file described in sub-section 8.2.1.

If you change to the `ProbABEL/examples` directory you can run an analysis of height by running

```

user@server:~/ProbABEL/examples/$ ../bin/palinear -p height.txt
-d test.mldose -i test.mlinfo

```

Output from the analysis will be directed to the `regression.out.csv` file.

The analysis of a binary trait (e.g. `chd`) can be run with

```

user@server:~/ProbABEL/examples/$ ../bin/palogist -p logist_data.txt
-d test.mldose -i test.mlinfo

```

To run a Cox proportional hazards model, try

```

user@server:~/ProbABEL/examples/$ ../bin/pacoxph -p coxph_data.txt
-d test.mldose -i test.mlinfo

```

Please have a look at the shell script files `example_qt.sh`, `example_bt.sh` and `example_all.sh` to have a better overview of the analysis options.

To run an analysis with MLPROB files, you need specify the MLPROB file with the `-d` option and also specify that there are two genetic predictors per SNP, e.g. you can run linear model with

```

user@server:~/ProbABEL/examples/$ ../bin/palinear -p height.txt
-d test.mlprob -i test.mlinfo
--ngpreds=2

```



### 8.3.2 Advanced analysis options

The option `-interaction` allows you to include interaction between SNPs and any covariate. If for example your model is

$$\text{trait} \sim \text{sex} + \text{age} + \text{SNP},$$

running the program with the option `-interaction=2` will model

$$\text{trait} \sim \text{sex} + \text{age} + \text{SNP} + \text{age} \times \text{SNP}.$$

The option `-robust` allows you to compute so-called “robust” (a.k.a. “sandwich”, a.k.a. Hubert-White) standard errors (cf. section 8.7 “Methodology” for details).

With the option `-mmscore` a score test for association between a trait and genetic polymorphisms in samples of related individuals is performed. A file with the inverse of the variance-covariance matrix goes as input parameter with that option, e.g. `-mmscore <filename>`. The file has to contain the first column with id names exactly like in phenotype file, BUT OMITTING people with no measured phenotype. The rest is a matrix. The phenotype file in case of using the `-mmscore` argument may contain any amount of covariates (this is different from previous versions). The first column contains id names, the second the trait. The others are covariates.

An example of how a polygenic object estimated by **GenABEL**-package can be used with **ProbABEL** is provided in `ProbABEL/examples/mmscore.R`

Though technically `-mmscore` allows for inclusion of multiple covariates, these should be kept to minimum as this is a score test. We suggest that any covariates explaining an essential proportion of variance should be fit as part of **GenABEL**-package’s polygenic procedure.

### 8.3.3 Running multiple analyses at once: `probabel.pl`

The Perl script `bin/probabel.pl_example` represents a handy wrapper for **ProbABEL**-package functions. To start using it the configuration file `bin/probabel_config.cfg_example` needs to be edited. The configuration file consists of five columns. Each column except the first is a pattern for files produced by **MACH** (imputation software). The column named “cohort” is an identifying name of a population (“ERGO” in this example), the column “mlinfo\_path” is the full path to mlinfo files, including a pattern where the chromosome number has been replaced by `._chr._.`. The columns “mldose\_path”, “mlprobe\_path” and “legend\_path” are paths and patterns for “mldose”, “mlprob” and “legend” files, respectively. These also need to include the pattern for the chromosome as used in the column for the “mlinfo” files. Probably you also have to change the variable `$config` in the script to point to the full path of the configuration file and the variable `@anprog` to point full path to the **ProbABEL**-package scripts.

## 8.4 Output file format

Let us consider what comes out of the linear regression analysis described in the previous section. After the analysis has run, in the output file you will find something like

```

name A1 A2 Freq1 MAF Quality Rsq n Mean_predictor_allele chrom
+> position beta_SNP_add sebeta_SNP_add chi2_SNP
rs7247199 G A 0.5847 0.415 0.9299 0.8666 182 0.564439 19
+> 204938 -0.218693 0.734966 0.0905063
rs8102643 C T 0.5847 0.415 0.9308 0.8685 182 0.564412 19
+> 207859 -0.218352 0.734214 0.0904094

```

Here, only the first three lines of output have been shown. Note that lines starting with `+>` are actually the ones continuing the previous line – they have just been wrapped so we can see these long lines.

The header provides a short description of what can be found in a specific column. The first column provides the SNP name and next six are descriptions which were taken directly from the SNP information file. Therefore, these describe allele frequencies and the quality in your total imputations, not necessarily in the data under analysis.

In contrast, starting with the next column, named `n`, the output concerns the data analysed. Column 8 (`n`) tells the number of subjects for whom complete phenotypic information was available. At this point, unless you have complete measurements on all subjects, you should feel alarmed if the number here is exactly the number of people in the file – this may indicate you did not code missing values according to **ProbABEL-package** format (`'NA'`, `'NaN'`, or `'N'`).

The next column, nine (“Mean\_predictor\_allele”), gives the estimated frequency of the predictor allele (`A1`) in subjects with complete phenotypic data.

If the `-chrom` option was used, in the next column you will find the value specified by this option. If `-map` option was used, in the subsequent column you will find map location taken from the map-file. The subsequent columns provide coefficients of regression of the phenotype onto genotype, corresponding standard errors, and Wald  $\chi^2$  test value.

## 8.5 Preparing input files

In the **ProbABEL/bin** directory you can find the `prepare_data.R` file – an R script that arranges phenotypic data in right format. Please read this script for details.

## 8.6 Memory use and performance

Maximum likelihood regression is implemented in **ProbABEL-package**. With 6,000 people and 2.5 millions SNPs, a genome-wide scan is completed in less than an hour for a linear model with 1-2 covariates and overnight for logistic regression or the Cox proportional hazards model (figures for a PC bought back in 2007).

Memory may be an issue with **ProbABEL-package** if you use MACH text dose/probability files, e.g. for large chromosomes, such as chromosome one consumed up to 5 GB of RAM with 6,000 people.

We suggest that dose/probability file is to be supplied in filevector format in which case **ProbABEL-package** will operate about 2-3 times faster, and in low-RAM mode (approx. 128 MB). See the R libraries **GenABEL-package** and

**DatABEL-package** on how to convert MACH and IMPUTE files to filevector format (functions: `mach2databel()` and `impute2databel()`, respectively).

When '-mmscore' option is used, the analysis may take quite some time.

## 8.7 Methodology

### 8.7.1 Analysis of population-based data

#### Linear regression assuming normal distribution

Standard linear regression theory is used to estimate coefficients of regression and their standard errors. We assume a linear model with expectation

$$E[\mathbf{Y}] = \mathbf{X}\boldsymbol{\beta} \quad (8.1)$$

and variance-covariance matrix

$$\mathbf{V} = \sigma^2 \mathbf{I},$$

where  $\mathbf{Y}$  is the vector of phenotypes of interest,  $\mathbf{X}$  is the design matrix,  $\boldsymbol{\beta}$  is the vector of regression parameters,  $\sigma^2$  is the variance and  $\mathbf{I}$  is the identity matrix.

The maximum likelihood estimates (MLEs) for the regression parameters are given by

$$\hat{\boldsymbol{\beta}} = (\mathbf{X}^T \mathbf{X})^{-1} \mathbf{X}^T \mathbf{Y} \quad (8.2)$$

and the MLE of the residual variance is

$$\hat{\sigma}^2 = \frac{(\mathbf{Y} - \mathbf{X}\hat{\boldsymbol{\beta}})^T (\mathbf{Y} - \mathbf{X}\hat{\boldsymbol{\beta}})}{N - r_X}, \quad (8.3)$$

where  $N$  is the number of observations and  $r_X$  is the rank of  $\mathbf{X}$  (i.e. the number of columns of the design matrix).

The variance-covariance matrix for the parameter estimates under alternative hypothesis can be computed as

$$\mathbf{var}_{\hat{\boldsymbol{\beta}}} = \hat{\sigma}^2 (\mathbf{X}^T \mathbf{X})^{-1}. \quad (8.4)$$

For the  $j$ -th element  $\hat{\beta}(j)$  of the vector of estimates the standard error under the alternative hypothesis is given by the square root of the corresponding diagonal element of the above matrix,  $\mathbf{var}_{\hat{\boldsymbol{\beta}}}(jj)$ , and the Wald test can be computed with

$$T^2(j) = \frac{\hat{\beta}(j)^2}{\mathbf{var}_{\hat{\boldsymbol{\beta}}}(jj)},$$

which asymptotically follows the  $\chi^2$  distribution with one degree of freedom under the null hypothesis.

When testing significance for more than one parameter simultaneously, several alternatives are available. Let us first partition the vector of parameters into two components,  $\boldsymbol{\beta} = (\boldsymbol{\beta}_g, \boldsymbol{\beta}_x)$ , and our interest is testing the parameters contained in  $\boldsymbol{\beta}_g$  (SNP effects), while  $\boldsymbol{\beta}_x$  (e.g. effects of sex, age, etc.) are considered nuisance parameters. Let us define the vector of the parameters of interest which are fixed to certain values under the null hypothesis as  $\boldsymbol{\beta}_{g,0}$ .

Firstly, the likelihood ratio test can be obtained with

$$LRT = 2 \left( \log \text{Lik}(\hat{\beta}_g, \hat{\beta}_x) - \log \text{Lik}(\beta_{g,0}, \hat{\beta}_x) \right),$$

which under the null hypothesis is asymptotically distributed as  $\chi^2$  with the number of degrees of freedom equal to the number of parameters specified by  $\beta_g$ . Assuming the normal distribution, the log-likelihood of a model specified by the vector of parameters  $\beta$  and residual variance  $\sigma^2$  can be computed as

$$\log \text{Lik}(\beta, \sigma^2) = -\frac{1}{2} \left( N \cdot \log_e \sigma^2 + (\mathbf{Y} - \beta \mathbf{X})^T (\mathbf{I}/\sigma^2) (\mathbf{Y} - \beta \mathbf{X}) \right).$$

Secondly, the Wald test can be used; for that the inverse variance-covariance matrix of  $\hat{\beta}_g$  should be computed as

$$\mathbf{var}_{\hat{\beta}_g}^{-1} = \mathbf{var}_{\hat{\beta}}^{-1}(g, g) - \mathbf{var}_{\hat{\beta}}^{-1}(g, x) \left( \mathbf{var}_{\hat{\beta}}^{-1}(x, x) \right)^{-1} \mathbf{var}_{\hat{\beta}}^{-1}(x, g),$$

where  $\mathbf{var}_{\hat{\beta}}^{-1}(a, b)$  correspond to sub-matrices of the inverse of the variance-covariance matrix of  $\hat{\beta}$ , involving either only parameters of interest  $(g, g)$ , nuisance parameters  $(x, x)$  or combination of these  $(x, g)$ ,  $(g, x)$ .

The Wald test statistics is then computed as

$$W^2 = (\hat{\beta}_g - \beta_{g,0})^T \mathbf{var}_{\hat{\beta}_g}^{-1} (\hat{\beta}_g - \beta_{g,0}),$$

which asymptotically follows the  $\chi^2$  distribution with the number of degrees of freedom equal to the number of parameters specified by  $\beta_g$ . The Wald test generally is computationally easier than the LRT, because it avoids estimation of the model specified by the parameter's vector  $(\beta_{g,0}, \hat{\beta}_x)$ .

Lastly, similar to the Wald test, the score test can be performed by use of  $\mathbf{var}_{(\beta_{g,0}, \hat{\beta}_x)}$  instead of  $\mathbf{var}_{\hat{\beta}}$ .

### Logistic regression

For logistic regression, the procedure to obtain parameters estimates, their variance-covariance matrix, and tests are similar to these outlined above with several modifications.

The expectation of the binary trait is defined as the expected probability of the event as defined by the logistic function

$$E[\mathbf{Y}] = \pi = \frac{1}{1 + e^{-(\mathbf{X}\beta)}}.$$

The estimates of the parameters are obtained not in one step, as is the case of the linear model, but using an iterative procedure (iteratively re-weighted least squares). This procedure is not described here for the sake of brevity.

The log-likelihood of the data is computed using the binomial probability formula:

$$\log \text{Lik}(\beta) = \mathbf{Y}^T \log_e \pi + (\mathbf{1} - \mathbf{Y})^T \log_e (\mathbf{1} - \pi),$$

where  $\log_e \pi$  is a vector obtained by taking the natural logarithm of every value contained in the vector  $\pi$ .

### Robust variance-covariance matrix of parameter estimates

For a linear model, these are computed using formula

$$\mathbf{var}_r = (\mathbf{X}^T \mathbf{X})^{-1} (\mathbf{X}^T \mathbf{R} \mathbf{X}) (\mathbf{X}^T \mathbf{X})^{-1},$$

where  $\mathbf{R}$  is a diagonal matrix containing squares of residuals of  $\mathbf{Y}$ . The same formula may be used for “standard” analysis, in which case the elements of the  $\mathbf{R}$  matrix are constant, namely mean residual sum of squares (the estimate of  $\sigma^2$ ).

Similar to that, the robust matrix is computed for logistic regression with

$$\mathbf{var}_r = (\mathbf{X}^T \mathbf{W} \mathbf{X})^{-1} (\mathbf{X}^T \mathbf{R} \mathbf{X}) (\mathbf{X}^T \mathbf{W} \mathbf{X})^{-1},$$

where  $\mathbf{1}$  is the vector of ones and  $\mathbf{W}$  is the diagonal matrix of “weights” used in logistic regression.

### Cox proportional hazards model

The implementation of the Cox proportional hazard model used in **ProbABEL-package** is entirely based on the code of R library **survival** developed by Thomas Lumley (function **coxfit2**), and is therefore not described here.

Many thanks to Thomas for making his code available under GNU GPL!

## 8.7.2 Analysis of pedigree data

The framework for analysis of pedigree data follows the two-step logic developed in the works of Aulchenko *et al.* (2007) and Chen and Abecasis (2007). General analysis model is a linear mixed model which defines the expectation of the trait as

$$E[\mathbf{Y}] = \mathbf{X}\beta,$$

identical to that defined for linear model (cf. section 8.1). To account for correlations between the phenotypes of relatives which may be induced by family relations the variance-covariance matrix is defined to be proportional to the linear combination of the identity matrix  $\mathbf{I}$  and the relationship matrix  $\Phi$ :

$$\mathbf{V}_{\sigma^2, h^2} = \sigma^2 (2h^2 \Phi + (1 - h^2) \mathbf{I}),$$

where  $h^2$  is the heritability of the trait. The relationship matrix  $\Phi$  is twice the matrix containing the coefficients of kinship between all pairs of individuals under consideration; its estimation is discussed in a separate section “8.7.2” (8.7.2).

Estimation of a model defined in such a way is possible by numerical maximization of the likelihood function, however, the estimation of this model for large pedigrees is laborious, and is not computationally feasible for hundreds of thousands to millions of SNPs to be tested in the context of GWAS, as we have demonstrated previously (Aulchenko *et al.*, 2007).

**Two-step score test for association**

A two-step score test approach is therefore used to decrease the computational burden. Let us first re-define the expectation of the trait by splitting the design matrix in two parts, the "base" part  $\mathbf{X}_x$ , which includes all terms not changing across all SNP models fit in GWAS (e.g. effects of sex, age, etc.), and the part including SNP information,  $\mathbf{X}_g$ :

$$E[\mathbf{Y}] = \mathbf{X}_x\beta_x + \mathbf{X}_g\beta_g.$$

Note that the latter design matrix may include not only the main SNP effect, but e.g. SNP by environment interaction terms.

At the first step, a linear mixed model not including SNP effects

$$E[\mathbf{Y}] = \mathbf{X}_x\beta_x$$

is fitted. The maximum likelihood estimates (MLEs) of the model parameters (regression coefficients for the fixed effects  $\hat{\beta}_x$ , the residual variance  $\hat{\sigma}_x^2$  and the heritability  $\hat{h}_x^2$ ) can be obtained by numerical maximization of the likelihood function

$$\log\text{Lik}(\beta_x, h^2, \sigma^2) = -\frac{1}{2} \left( \log_e |\mathbf{V}_{\sigma^2, h^2}| + (\mathbf{Y} - \beta_x \mathbf{X}_x)^T \mathbf{V}_{\sigma^2, h^2}^{-1} (\mathbf{Y} - \beta_x \mathbf{X}_x) \right),$$

where  $\mathbf{V}_{\sigma^2, h^2}^{-1}$  is the inverse and  $|\mathbf{V}_{\sigma^2, h^2}|$  is the determinant of the variance-covariance matrix.

At the second step, the unbiased estimates of the fixed effects of the terms involving SNP are obtained with

$$\hat{\beta}_g = (\mathbf{X}_g^T \mathbf{V}_{\hat{\sigma}^2, \hat{h}^2}^{-1} \mathbf{X}_g)^{-1} \mathbf{X}_g^T \mathbf{V}_{\hat{\sigma}^2, \hat{h}^2}^{-1} \mathbf{R}_{\hat{\beta}_x},$$

where  $\mathbf{V}_{\hat{\sigma}^2, \hat{h}^2}^{-1}$  is the variance-covariance matrix at the point of the MLE estimates of  $\hat{h}_x^2$  and  $\hat{\sigma}_x^2$  and  $\mathbf{R}_{\hat{\beta}_x} = \mathbf{Y} - \hat{\beta}_x \mathbf{X}_x$  is the vector of residuals obtained from the base regression model. Under the null model, the inverse variance-covariance matrix of the parameter's estimates is defined as

$$\mathbf{var}_{\hat{\beta}_g} = \hat{\sigma}_x^2 (\mathbf{X}_g^T \mathbf{V}_{\hat{\sigma}^2, \hat{h}^2}^{-1} \mathbf{X}_g)^{-1}.$$

Thus the score test for joint significance of the terms involving SNP can be obtained with

$$T^2 = (\hat{\beta}_g - \beta_{g,0})^T \mathbf{var}_{\hat{\beta}_g}^{-1} (\hat{\beta}_g - \beta_{g,0}),$$

where  $\beta_{g,0}$  are the values of parameters fixed under the null model. This test statistics under the null hypothesis asymptotically follows the  $\chi^2$  distribution with the number of degrees of freedom equal to the number of parameters tested. The significance of an individual  $j$ -the elements of the vector  $\hat{\beta}_g$  can be tested with

$$T_j^2 = \hat{\beta}_g^2(j) \mathbf{var}_{\hat{\beta}_g}^{-1}(jj),$$

where  $\hat{\beta}_g^2(j)$  is the square of the  $j$ -th element of the vector of estimates  $\hat{\beta}_g$ , and  $\mathbf{var}_{\hat{\beta}_g}^{-1}(jj)$  corresponds to the  $j$ -th diagonal element of  $\mathbf{var}_{\hat{\beta}_g}^{-1}$ . The latter statistics asymptotically follows  $\chi_1^2$ .

### Estimation of the kinship matrix

The relationship matrix  $\Phi$  used in estimation of the linear mixed model for pedigree data is twice the matrix containing the coefficients of kinship between all pairs of individuals under consideration. This coefficient is defined as the probability that two gametes randomly sampled from each member of the pair are identical-by-descent (IBD), that is they are copies of exactly the same ancestral allele. The expectation of kinship can be estimated from pedigree data using standard methods, for example the kinship for two outbred sibs is  $1/4$ , for grandchild-grandparent is  $1/8$ , etc. For an outbred person, the kinship coefficient is  $1/2$  – that is two gametes sampled from this person at random are IBD only if the same gamete is sampled. However, if the person is inbred, there is a chance that a maternal and paternal chromosomes are also IBD. The probability of this is characterized by kinship between individual's parents, which is defined as the individual's inbreeding coefficient,  $F$ . In this case, the kinship coefficient for the individual is  $F + 1/2$ . Similar logic applies to computation of the kinship coefficient for other types of pairs in inbred pedigrees.

The kinship matrix can be computed using the pedigree data using standard methods. However, in many cases, pedigree information may be absent, incomplete, or not reliable. Moreover, the estimates obtained using pedigree data reflect the expectation of the kinship, while the true realization of kinship may vary around this expectation. In presence of genomic data it may therefore be desirable to estimate the kinship coefficient from these, and not from pedigree. It can be demonstrated that unbiased and positive semi-definite estimator of the kinship matrix can be obtained (Astle and Balding, 2010; Amin *et al.*, 2007) by computing the kinship coefficients between individuals  $i$  and  $j$  with

$$\hat{K}_{ij} = \frac{1}{L} \sum_{l=1}^L \frac{(g_{l,i} - p_l)(g_{l,j} - p_l)}{p_l(1 - p_l)}$$

where  $L$  is the number of loci,  $p_l$  is the allelic frequency at  $l$ -th locus and  $g_{l,j}$  is the genotype of  $j$ -th person at the  $l$ -th locus, coded as 0,  $1/2$ , and 1, corresponding to the homozygous, heterozygous, and other type of homozygous genotype. The frequency is computed for the allele which, when homozygous, corresponds to the genotype coded as “1”.

## 8.8 How to cite

If you used ProbABEL-package for your analysis please give a link to the GenABEL project home page

<http://www.genabel.org/>

and cite the ProbABEL-package paper to give us some credit:

Aulchenko YS, Struchalin MV, van Duijn CM.  
*ProbABEL package for genome-wide association analysis of imputed data.*  
 BMC Bioinformatics. 2010, 11:134.

A proper reference may look like

For the analysis of imputed data, we used the **ProbABEL-package** from the **GenABEL** suite of programs (Aulchenko *et al.*, 2010).

If you have used the Cox proportional hazard model, please mention the R package **survival** by Thomas Lumley. Additionally to the above citation, please tell that

The Cox proportional hazards model implemented in **ProbABEL-package** makes use of the source code of the R package "**survival**" as implemented by T. Lumley.



## Chapter 9

# Analysis of imputed data: an example

In this chapter, you will perform an analysis of imputed data set. In this set of 120 individuals, 4500 SNPs are imputed based on information on 500 directly typed SNPs. You will first analyse 500 directly typed SNPs and then proceed to the analysis of imputed data. Finally, you will have a possibility to compare your results to the results of analysis in case all 5000 SNPs were directly typed.

### 9.1 Analysis of 500 directly typed SNPs

Load the **GenABEL**-package library and load the data set we will use:

```
> library(GenABEL)
> load("RData/ImputedDataAnalysis.RData")
> ls()
```

```
[1] "df500" "df5k"  "map5k" "old"   "rcT"
```

Here, **df500** contains the data including 500 directly typed SNPs, and **rcT** is the vector containing the value of the trait of interest:

```
> nids(df500)
```

```
[1] 120
```

```
> nsnps(df500)
```

```
[1] 500
```

```
> length(rcT)
```

```
[1] 120
```

```
> rcT[1:10]
```

```
[1] -0.5560057  1.1798113  0.2721125  0.2174227 -0.8193852 -1.8739403
[7]  0.9530623  0.9022811 -1.0906900  0.5575454
```

In all analysis that follow, do disregard the Genomic Control and GC-corrected results: as we will analyse a small region with strong association, the GC can not be applied.

Let us start with analysis of directly typed SNPs. For that, we will use `mlreg` function of **GenABEL**-package. This function implements ML-regression and Wald test of significance<sup>1</sup>. This will later on allow us direct comparison with the results of **ProbABEL**-package, which implements the same testing procedure.

We can run regression of `rcT` on SNPs region-wise using

```
> qts500 <- mlreg(rcT~1,df500)
> qts500[1:5,]
```

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB
rs6139074	20	11244	+	A	C	120	0.488853649	0.2079789
rs13043000	20	13288	+	G	T	120	-0.002890489	0.4394487
rs6037629	20	43093	+	T	G	120	0.504885204	0.4040614
rs6052070	20	44931	+	A	G	120	0.171428327	0.3037772
rs6116135	20	46930	+	G	A	120	-0.239668333	0.4235491

	chi2.1df	P1df	Pc1df	effAB	effBB	chi2.2df	P2df
rs6139074	5.524833e+00	0.0187484	0.2123725	NA	NA	NA	NA
rs13043000	4.326395e-05	0.9947519	0.9972156	NA	NA	NA	NA
rs6037629	1.561315e+00	0.2114728	0.5073673	NA	NA	NA	NA
rs6052070	3.184599e-01	0.5725346	0.7646316	NA	NA	NA	NA
rs6116135	3.201945e-01	0.5714908	0.7640104	NA	NA	NA	NA

The summary for the SNPs, which show most significant association can be produced with

```
> bestHits500 <- descriptives.scan(qts500,top=10)
```

Summary for top 10 results, sorted by P1df

```
> bestHits500
```

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB	chi2.1df
rs6039167	20	846271	+	G	A	120	-5.9451984	0.7731855	59.12420
rs7261762	20	853448	+	A	G	120	-6.7335160	0.9748760	47.70732
rs511582	20	868752	+	A	G	120	-0.9872044	0.1883796	27.46290
rs967789	20	2876445	+	A	G	120	-0.9289317	0.1949364	22.70814
rs642758	20	2831146	+	A	G	120	-0.9194347	0.1993352	21.27521
rs676749	20	2974069	+	A	T	120	-0.8711208	0.1900255	21.01517
rs577116	20	2818285	+	G	A	120	-0.9291733	0.2032178	20.90595
rs570673	20	2819015	+	C	G	120	-0.8728347	0.1973862	19.55377
rs6112914	20	2048348	+	A	G	120	-0.6753674	0.1543434	19.14715
rs6106161	20	1995179	+	T	G	120	-0.6253343	0.1609140	15.10208

	P1df	Pc1df	effAB	effBB	chi2.2df	P2df
rs6039167	1.480274e-14	4.512105e-05	NA	NA	NA	NA
rs7261762	4.948399e-12	2.477531e-04	NA	NA	NA	NA
rs511582	1.601368e-07	5.429559e-03	NA	NA	NA	NA
rs967789	1.885672e-06	1.146299e-02	NA	NA	NA	NA

<sup>1</sup> In general the score test may be preferred because it is faster and more robust

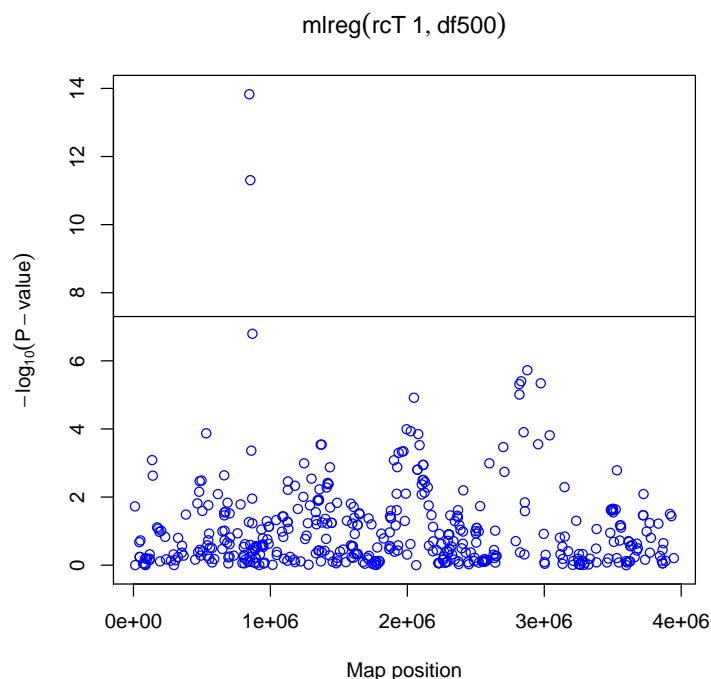


Figure 9.1: Manhattan plot for 500 directly typed SNPs

rs642758	3.978434e-06	1.439751e-02	NA	NA	NA	NA
rs676749	4.556610e-06	1.500792e-02	NA	NA	NA	NA
rs577116	4.823944e-06	1.527219e-02	NA	NA	NA	NA
rs570673	9.780761e-06	1.897119e-02	NA	NA	NA	NA
rs6112914	1.210181e-05	2.025583e-02	NA	NA	NA	NA
rs6106161	1.018509e-04	3.922563e-02	NA	NA	NA	NA

and the plot of the results with

```
> plot(qts500)
> abline(h=-log10(5e-8))
```

(see figure 9.1).

Finally, we can produce descriptive statistics for the SNPs, which demonstrated genome-wide significance:

```
> gwsSnps500 <- rownames(bestHits500)[bestHits500$P1df<=6e-8]
> gwsSnps500
```

```
[1] "rs6039167" "rs7261762"
```

```
> summary(gtdata(df500[,gwsSnps500 ]))
```

	Chromosome	Position	Strand	A1	A2	NoMeasured	CallRate	Q.2	P.11
rs6039167	20	846271	+	G	A	120	1 0.012500000	117	

rs7261762	20	853448	+	A	G	120	1	0.008333333	118
	P.12	P.22	Pexact		Fmax		Plrt		
rs6039167	3	0	1	-0.012658228	0.8454919				
rs7261762	2	0	1	-0.008403361	0.8968501				

### Exercise 1.

It is known that rare variation in presence of outliers can generate spurious associations. Do you believe this is true association in this particular case? What you can do to check whether this is true association or not?

## 9.2 Analysis of imputed data with ProbABEL-package

Here, you will analyse imputed data. In `RData` directory, you will find the necessary files: `mach1.mldose.fvi` and `mach1.mldose.fvd` (these files represent `mldose` data produced by `mach1`, converted into `DatABEL-package` format<sup>2</sup>) and `mach1.out.mlinfo`, which contains information for the imputed SNPs generated in the process of imputations (such as 'Rsq', etc.).

We will start with producing a phenotypic data file for the use with `ProbABEL-package`:

```
> write.table(data.frame(id=idnames(df500),rcT=rcT),
+             file="rcT.PHE",quote=F,row.names=F)
```

next, try command `'system("head rcT.PHE")'` to check few first lines of the file.

At this moment, leave R (or, rather, start new console!), copy the `mach`-files to the working directory with

```
yourname@server> cp RData/mach1* .
```

and run `ProbABEL-package` analysis with

```
yourname@server> palinear --pheno rcT.PHE --info mach1.out.mlinfo /
--dose mach1.mldose.fvi
```

Do not forget to check that you start analysis in right directory, i.e. all files (`rcT.PHE`, `mach1.out.mlinfo`, `mach1.mldose.fvi`, `mach1.mldose.fvd`) are present in the working directory (use command `'ls'` from the console).

Now, you can return to R and load the analysis results:

```
> qtsPal <- read.table("regression_add.out.txt",head=T,strings=F)
> qtsPal[1:5,]
```

	name	A1	A2	Freq1	MAF	Quality	Rsq	n	Mean_predictor_allele
1	rs4814683	T	G	0.4921	0.4921	0.7334	0.5650	120	0.492087
2	rs6076506	T	G	0.7723	0.2277	0.6511	0.2415	120	0.772271
3	rs6139074	C	A	0.3384	0.3384	0.9904	0.9803	120	0.338387
4	rs1418258	T	C	0.5009	0.4991	0.7208	0.5474	120	0.500917
5	rs7274499	C	A	0.9745	0.0255	0.9505	0.1011	120	0.974450

<sup>2</sup>using `mach2databel`

	beta_SNP_add	sebeta_SNP_add	loglik
1	0.618307	0.269166	-114.266
2	0.722713	0.499781	-115.837
3	0.501472	0.210870	-114.082
4	0.636760	0.273391	-114.194
5	1.348270	2.085770	-116.679

As you see, there is not *P*-value produced in the ProbABEL-package output, and we need to compute it:

```
> qtsPal$Chisq <- (qtsPal$beta/qtsPal$sebeta)^2
> qtsPal$"P-value" <- pchisq(qtsPal$Chisq,1,low=F)
> qtsPal[1:5,]
```

	name	A1	A2	Freq1	MAF	Quality	Rsq	n	Mean_predictor_allele
1	rs4814683	T	G	0.4921	0.4921	0.7334	0.5650	120	0.492087
2	rs6076506	T	G	0.7723	0.2277	0.6511	0.2415	120	0.772271
3	rs6139074	C	A	0.3384	0.3384	0.9904	0.9803	120	0.338387
4	rs1418258	T	C	0.5009	0.4991	0.7208	0.5474	120	0.500917
5	rs7274499	C	A	0.9745	0.0255	0.9505	0.1011	120	0.974450

	beta_SNP_add	sebeta_SNP_add	loglik	Chisq	P-value
1	0.618307	0.269166	-114.266	5.2767671	0.02161184
2	0.722713	0.499781	-115.837	2.0910877	0.14816055
3	0.501472	0.210870	-114.082	5.6554059	0.01740165
4	0.636760	0.273391	-114.194	5.4247924	0.01985280
5	1.348270	2.085770	-116.679	0.4178505	0.51801156

Let us have a look at the top 20 associated SNPs:

```
> qtsPal[order(qtsPal$Chisq,decreasing=T)[1:20],]
```

	name	A1	A2	Freq1	MAF	Quality	Rsq	n	Mean_predictor_allele
1009	rs6039167	G	A	0.9875	0.0125	0.9995	0.9800	120	0.987537
1022	rs7273309	C	T	0.9985	0.0015	0.9970	0.1896	120	0.998550
1026	rs7265788	A	G	0.9886	0.0114	0.9929	0.6884	120	0.988546
1025	rs8123328	G	T	0.9902	0.0098	0.9964	0.8126	120	0.990246
1024	rs7267882	G	A	0.9911	0.0089	0.9986	0.9230	120	0.991167
4009	rs566570	C	T	0.5307	0.4693	0.8814	0.8074	120	0.530712
1023	rs7261762	A	G	0.9913	0.0087	0.9991	0.9472	120	0.991346
2973	rs6035871	A	G	0.9387	0.0613	0.8962	0.2582	120	0.938696
2969	rs6047425	A	G	0.9388	0.0612	0.8964	0.2573	120	0.938767
3877	rs2325971	T	C	0.5811	0.4189	0.8526	0.7807	120	0.581042
1017	rs553378	G	A	0.9987	0.0013	0.9974	0.0005	120	0.998704
3880	rs873711	A	G	0.5888	0.4112	0.8597	0.7934	120	0.588750
3886	rs6037425	G	C	0.5871	0.4129	0.8651	0.8034	120	0.587125
3958	rs6037443	A	G	0.4975	0.4975	0.8918	0.8340	120	0.497446
4027	rs6076466	T	C	0.7040	0.2960	0.6570	0.3365	120	0.703938
3884	rs6051434	G	T	0.5186	0.4814	0.8427	0.7732	120	0.518621
1018	rs554362	A	G	0.6496	0.3504	0.5748	0.2481	120	0.649604
4032	rs2326056	C	A	0.7338	0.2662	0.6422	0.2403	120	0.733767
4029	rs11697448	G	A	0.7414	0.2586	0.6464	0.2370	120	0.741396

```

1560 rs6033304 A C 0.8234 0.1766 0.7408 0.2813 120 0.823429
      beta_SNP_add sebeta_SNP_add loglik Chisq P-value
1009 5.98851 0.781065 -92.6360 58.78455 1.759171e-14
1022 38.75330 5.101300 -93.0016 57.71061 3.036607e-14
1026 7.39562 0.977268 -93.1526 57.26929 3.800428e-14
1025 7.12447 0.984098 -94.8389 52.41172 4.500298e-13
1024 6.85041 0.977608 -96.0155 49.10250 2.429299e-12
4009 1.29428 0.185797 -96.2227 48.52651 3.258536e-12
1023 6.80755 0.977629 -96.2366 48.48792 3.323302e-12
2973 4.88622 0.721348 -97.1826 45.88348 1.254997e-11
2969 4.89489 0.722872 -97.1940 45.85251 1.275000e-11
3877 1.29728 0.192726 -97.3933 45.30925 1.682530e-11
1017 728.01600 108.683000 -97.5547 44.87026 2.105310e-11
3880 1.26975 0.192071 -97.9861 43.70316 3.821541e-11
3886 1.25994 0.190625 -97.9929 43.68577 3.855647e-11
3958 1.21385 0.184428 -98.1290 43.31879 4.650954e-11
4027 1.98273 0.339011 -101.6180 34.20579 4.958132e-09
3884 1.14070 0.199652 -102.2370 32.64341 1.107140e-08
1018 2.12435 0.376830 -102.5820 31.78053 1.726140e-08
4032 2.33945 0.427909 -103.3440 29.88992 4.572850e-08
4029 2.36276 0.435773 -103.5440 29.39805 5.893679e-08
1560 2.39452 0.451563 -104.0670 28.11902 1.140790e-07

```

and produce the plot of the results with

```

> plot(map5k, -log10(qtsPal[, "P-value"]))
> abline(h=-log10(5e-8))

```

(here, `map5k` contains map information for all 5000 SNPs).

To compare with the results obtained using 500 directly typed SNPs only, we can add the points with

```

> points(map(qts500), -log10(qts500[, "P1df"]), col="red", pch=19, cex=0.5)

```

(see figure 9.2).

Now, we can also check different characteristics of the SNPs, which are claimed to be significant in our analysis:

```

> gwsSnpsImp <- qtsPal$name[which(qtsPal[, "P-value"] <= 5e-8)]
> gwsSnpsImp

[1] "rs6039167" "rs553378" "rs554362" "rs7273309" "rs7261762" "rs7267882"
[7] "rs8123328" "rs7265788" "rs6047425" "rs6035871" "rs2325971" "rs873711"
[13] "rs6051434" "rs6037425" "rs6037443" "rs566570" "rs6076466" "rs2326056"

> mlinfo <- read.table("mach1.out.mlinfo", head=T, strings=F)
> mlinfo[mlinfo$SNP %in% gwsSnpsImp,]

```

```

      SNP A11 A12 Freq1 MAF Quality Rsq
1009 rs6039167 G A 0.9875 0.0125 0.9995 0.9800
1017 rs553378 G A 0.9987 0.0013 0.9974 0.0005
1018 rs554362 A G 0.6496 0.3504 0.5748 0.2481

```

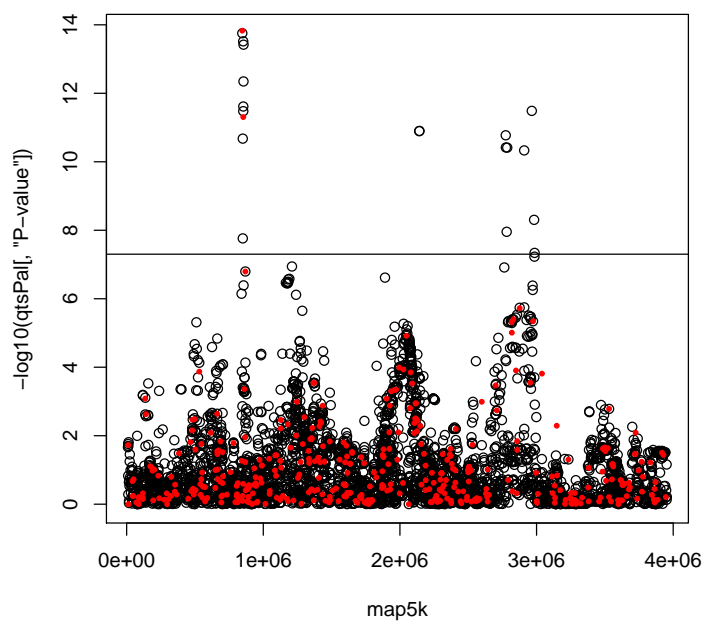


Figure 9.2: Manhattan plot for imputed (black empty circles) and 500 directly typed (red solid circles) SNPs

1022	rs7273309	C	T	0.9985	0.0015	0.9970	0.1896
1023	rs7261762	A	G	0.9913	0.0087	0.9991	0.9472
1024	rs7267882	G	A	0.9911	0.0089	0.9986	0.9230
1025	rs8123328	G	T	0.9902	0.0098	0.9964	0.8126
1026	rs7265788	A	G	0.9886	0.0114	0.9929	0.6884
2969	rs6047425	A	G	0.9388	0.0612	0.8964	0.2573
2973	rs6035871	A	G	0.9387	0.0613	0.8962	0.2582
3877	rs2325971	T	C	0.5811	0.4189	0.8526	0.7807
3880	rs873711	A	G	0.5888	0.4112	0.8597	0.7934
3884	rs6051434	G	T	0.5186	0.4814	0.8427	0.7732
3886	rs6037425	G	C	0.5871	0.4129	0.8651	0.8034
3958	rs6037443	A	G	0.4975	0.4975	0.8918	0.8340
4009	rs566570	C	T	0.5307	0.4693	0.8814	0.8074
4027	rs6076466	T	C	0.7040	0.2960	0.6570	0.3365
4032	rs2326056	C	A	0.7338	0.2662	0.6422	0.2403

### Exercise 2.

Please classify the associations obtained into 'true' and 'false' (meaning 'I believe it' and 'I do not believe'). Of course ultimate answer is replications. Still, the object `df5k` provides genotypes for all 5000 SNPs, directly typed! So,

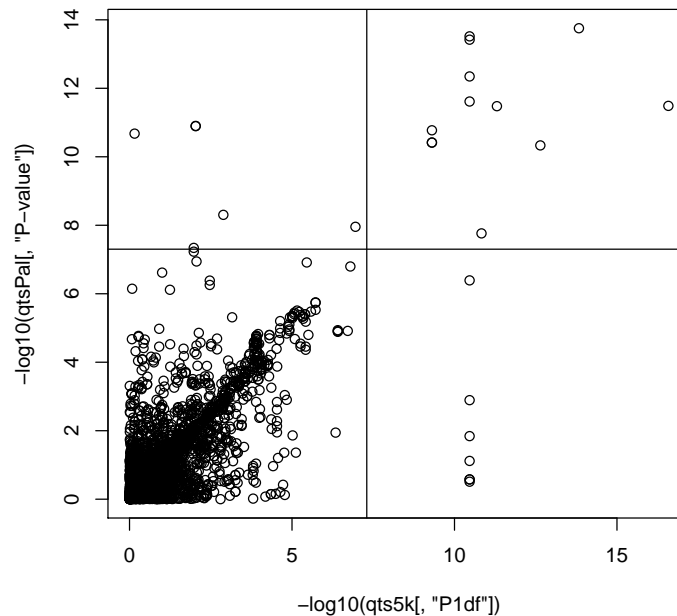


Figure 9.3: Cross-plot of the results from analysis of imputed and directly typed data (see answers to exercises).

you can run analysis, and cross-check which SNPs are confirmed as significant. Make a cross table: SNPs you thought were truly associated vs. SNPs indeed associated in directly typed data set.

### 9.3 Analysis of imputed data with MixABEL-package

In case you are interested in quantitative traits, **MixABEL-package** provides much faster and more flexible analysis tools, compared to the **ProbABEL-package**<sup>3</sup>.

Here the above analysis is repeated using **MixABEL-package**.

Load necessary libraries:

```
> library(DataABEL)
```

```
DataABEL v. 0.9-3 (February 09, 2011) loaded
```

Installed DataABEL version (0.9-3) is not the same as stable version available from CRAN (0.9-2). Unless used intentionally, consider updating to the latest CRAN version. For that, use `'install.packages("DataABEL")'`, or ask your system administrator to update the package.

<sup>3</sup>However, at the moment **MixABEL-package** misses functionality to analyse binary and time-till-event traits



```
> library(MixABEL)
```

```
MixABEL v 0.1-1 (February 23, 2011) loaded
```

Load the genotypic data:

```
> imp5k <- databel("mach1.mldose")
```

Run analysis and look up 10 top results:

```
> qtsImp <- GWFGSL(rcT~SNP,geno=imp5k,data=NULL)
> qtsImp[order(qtsImp[, "Chisq"],decreasing=T)[1:10],]
```

	n	Chisq	df	P-value	beta_SNP	se_SNP	mean_SNP
rs6039167	120	58.78462	1	1.759110e-14	5.988511	0.7810647	1.975075
rs7273309	120	57.71070	1	3.036458e-14	38.753300	5.1012957	1.997100
rs7265788	120	57.26926	1	3.800491e-14	7.395619	0.9772682	1.977092
rs8123328	120	52.41165	1	4.500458e-13	7.124468	0.9840984	1.980492
rs7267882	120	49.10249	1	2.429317e-12	6.850411	0.9776083	1.982333
rs566570	120	48.52652	1	3.258526e-12	1.294281	0.1857972	1.061425
rs7261762	120	48.48790	1	3.323324e-12	6.807552	0.9776294	1.982692
rs6035871	120	45.88351	1	1.254980e-11	4.886221	0.7213479	1.877392
rs6047425	120	45.85245	1	1.275033e-11	4.894889	0.7228723	1.877533
rs2325971	120	45.30891	1	1.682820e-11	1.297276	0.1927261	1.162083

Look up the information for SNPs with  $P$ -value less than  $6e-8$ :

```
> #plot(map5k,-log10(qtsImp[, "P-value"]))
> #abline(h=-log10(5e-8))
> gwsSnpsImp <- rownames(qtsImp)[which(qtsImp[, "P-value"]<=6e-8)]
> mlinfo <- read.table("mach1.out.mlinfo",head=T,strings=F)
> mlinfo[mlinfo$SNP %in% gwsSnpsImp,]
```

	SNP	Al1	Al2	Freq1	MAF	Quality	Rsq
1009	rs6039167	G	A	0.9875	0.0125	0.9995	0.9800
1017	rs553378	G	A	0.9987	0.0013	0.9974	0.0005
1018	rs554362	A	G	0.6496	0.3504	0.5748	0.2481
1022	rs7273309	C	T	0.9985	0.0015	0.9970	0.1896
1023	rs7261762	A	G	0.9913	0.0087	0.9991	0.9472
1024	rs7267882	G	A	0.9911	0.0089	0.9986	0.9230
1025	rs8123328	G	T	0.9902	0.0098	0.9964	0.8126
1026	rs7265788	A	G	0.9886	0.0114	0.9929	0.6884
2969	rs6047425	A	G	0.9388	0.0612	0.8964	0.2573
2973	rs6035871	A	G	0.9387	0.0613	0.8962	0.2582
3877	rs2325971	T	C	0.5811	0.4189	0.8526	0.7807
3880	rs873711	A	G	0.5888	0.4112	0.8597	0.7934
3884	rs6051434	G	T	0.5186	0.4814	0.8427	0.7732
3886	rs6037425	G	C	0.5871	0.4129	0.8651	0.8034
3958	rs6037443	A	G	0.4975	0.4975	0.8918	0.8340
4009	rs566570	C	T	0.5307	0.4693	0.8814	0.8074
4027	rs6076466	T	C	0.7040	0.2960	0.6570	0.3365
4029	rs11697448	G	A	0.7414	0.2586	0.6464	0.2370
4032	rs2326056	C	A	0.7338	0.2662	0.6422	0.2403

## 9.4 Answers to exercises

**Answer (Ex. 1)** — Firstly, you can check (by producing a cross-plot of genotype vs. phenotype) if association is indeed due to extreme phenotypic outliers. A related question is whether the distribution is skewed. Additionally, a permutation-based test can help establishing correct p-value, taking into account the nature of the data in question.

However, to give an ultimate answer, a replication study is needed, in which these rare SNPs are to be typed in a large independent sample.

**Answer (Ex. 2)** — Here is the sequence of commands leading you to the answer:

```
> qts5k <- mlreg(rcT~1,df5k)
> bestHits5k <- descriptives.scan(qts5k,top=20)
```

Summary for top 20 results, sorted by P1df

```
> bestHits5k
```

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB	chi2.1df
rs566570	20	2965113	+	T	C	120	1.341584	0.1585342	71.61267
rs6039167	20	846271	+	G	A	120	-5.945198	0.7731855	59.12420
rs6037443	20	2909408	+	G	A	120	1.194178	0.1628897	53.74655
rs7261762	20	853448	+	A	G	120	-6.733516	0.9748760	47.70732
rs554362	20	850002	+	A	G	120	-5.431215	0.8045925	45.56613
rs2104741	20	787070	+	G	A	120	-9.203756	1.3888630	43.91485
rs7273309	20	853154	+	C	T	120	-9.203756	1.3888630	43.91485
rs7267882	20	853785	+	G	A	120	-9.203756	1.3888630	43.91485
rs8123328	20	855045	+	G	T	120	-9.203756	1.3888630	43.91485
rs7265788	20	855426	+	A	G	120	-9.203756	1.3888630	43.91485
rs7263171	20	855655	+	C	T	120	-9.203756	1.3888630	43.91485
rs6110342	20	1458950	+	T	G	120	-9.203756	1.3888630	43.91485
rs6105340	20	1493635	+	G	A	120	-9.203756	1.3888630	43.91485
rs11905071	20	1557640	+	T	C	120	-9.203756	1.3888630	43.91485
rs6080013	20	1590861	+	G	A	120	-9.203756	1.3888630	43.91485
rs6074978	20	1595216	+	T	C	120	-9.203756	1.3888630	43.91485
rs2325971	20	2774477	+	T	C	120	-1.055991	0.1697536	38.69752
rs873711	20	2775468	+	A	G	120	-1.055991	0.1697536	38.69752
rs6037425	20	2785379	+	G	C	120	-1.055991	0.1697536	38.69752
rs6051434	20	2781237	+	T	G	120	0.938854	0.1769196	28.16076
	P1df	Pc1df	effAB	effBB	chi2.2df	P2df			
rs566570	2.618695e-17	5.551496e-06	NA	NA	NA	NA			
rs6039167	1.480274e-14	3.663781e-05	NA	NA	NA	NA			
rs6037443	2.280947e-13	8.301445e-05	NA	NA	NA	NA			
rs7261762	4.948399e-12	2.090409e-04	NA	NA	NA	NA			
rs554362	1.475718e-11	2.904523e-04	NA	NA	NA	NA			
rs2104741	3.429767e-11	3.745369e-04	NA	NA	NA	NA			
rs7273309	3.429767e-11	3.745369e-04	NA	NA	NA	NA			
rs7267882	3.429767e-11	3.745369e-04	NA	NA	NA	NA			
rs8123328	3.429767e-11	3.745369e-04	NA	NA	NA	NA			
rs7265788	3.429767e-11	3.745369e-04	NA	NA	NA	NA			

```

rs7263171 3.429767e-11 3.745369e-04 NA NA NA NA
rs6110342 3.429767e-11 3.745369e-04 NA NA NA NA
rs6105340 3.429767e-11 3.745369e-04 NA NA NA NA
rs11905071 3.429767e-11 3.745369e-04 NA NA NA NA
rs6080013 3.429767e-11 3.745369e-04 NA NA NA NA
rs6074978 3.429767e-11 3.745369e-04 NA NA NA NA
rs2325971 4.948386e-10 8.395801e-04 NA NA NA NA
rs873711 4.948386e-10 8.395801e-04 NA NA NA NA
rs6037425 4.948386e-10 8.395801e-04 NA NA NA NA
rs6051434 1.116452e-07 4.389549e-03 NA NA NA NA

```

```

> plot(qts5k)
> abline(h=-log10(5e-8))
> gwsSnps5k <- rownames(bestHits5k)[bestHits5k$P1df<=6e-8]
> summary(gtdata(df5k[,gwsSnps5k ]))

```

	Chromosome	Position	Strand	A1	A2	NoMeasured	CallRate	Q.2
rs566570	20	2965113	+	T	C	120	1	0.495833333
rs6039167	20	846271	+	G	A	120	1	0.012500000
rs6037443	20	2909408	+	G	A	120	1	0.491666667
rs7261762	20	853448	+	A	G	120	1	0.008333333
rs554362	20	850002	+	A	G	120	1	0.012500000
rs2104741	20	787070	+	G	A	120	1	0.004166667
rs7273309	20	853154	+	C	T	120	1	0.004166667
rs7267882	20	853785	+	G	A	120	1	0.004166667
rs8123328	20	855045	+	G	T	120	1	0.004166667
rs7265788	20	855426	+	A	G	120	1	0.004166667
rs7263171	20	855655	+	C	T	120	1	0.004166667
rs6110342	20	1458950	+	T	G	120	1	0.004166667
rs6105340	20	1493635	+	G	A	120	1	0.004166667
rs11905071	20	1557640	+	T	C	120	1	0.004166667
rs6080013	20	1590861	+	G	A	120	1	0.004166667
rs6074978	20	1595216	+	T	C	120	1	0.004166667
rs2325971	20	2774477	+	T	C	120	1	0.470833333
rs873711	20	2775468	+	A	G	120	1	0.470833333
rs6037425	20	2785379	+	G	C	120	1	0.470833333

	P.11	P.12	P.22	Pexact	Fmax	Plrt
rs566570	33	55	32	0.3648758	0.083269672	0.3614010
rs6039167	117	3	0	1.0000000	-0.012658228	0.8454919
rs6037443	35	52	33	0.1467972	0.133092526	0.1442654
rs7261762	118	2	0	1.0000000	-0.008403361	0.8968501
rs554362	117	3	0	1.0000000	-0.012658228	0.8454919
rs2104741	119	1	0	1.0000000	-0.004184100	0.9484250
rs7273309	119	1	0	1.0000000	-0.004184100	0.9484250
rs7267882	119	1	0	1.0000000	-0.004184100	0.9484250
rs8123328	119	1	0	1.0000000	-0.004184100	0.9484250
rs7265788	119	1	0	1.0000000	-0.004184100	0.9484250
rs7263171	119	1	0	1.0000000	-0.004184100	0.9484250
rs6110342	119	1	0	1.0000000	-0.004184100	0.9484250
rs6105340	119	1	0	1.0000000	-0.004184100	0.9484250
rs11905071	119	1	0	1.0000000	-0.004184100	0.9484250

```

rs6080013  119    1    0 1.0000000 -0.004184100 0.9484250
rs6074978  119    1    0 1.0000000 -0.004184100 0.9484250
rs2325971   38   51   31 0.1032427 0.147097763 0.1065638
rs873711    38   51   31 0.1032427 0.147097763 0.1065638
rs6037425   38   51   31 0.1032427 0.147097763 0.1065638

> plot(-log10(qts5k[, "P1df"]), -log10(qtsPal[, "P-value"]))
> abline(h=-log10(5e-8))
> abline(v=-log10(5e-8))
> directNotImp <- gwsSnps5k[!(gwsSnps5k %in% gwsSnpsImp)]
> directNotImp

[1] "rs2104741" "rs7263171" "rs6110342" "rs6105340" "rs11905071"
[6] "rs6080013" "rs6074978"

> imputeNotDir <- gwsSnpsImp[!(gwsSnpsImp %in% gwsSnps5k)]
> imputeNotDir

[1] "rs553378" "rs6047425" "rs6035871" "rs6051434" "rs6076466" "rs2326056"

> inBoth <- gwsSnps5k[gwsSnps5k %in% gwsSnpsImp]
> inBoth

[1] "rs566570" "rs6039167" "rs6037443" "rs7261762" "rs554362" "rs7273309"
[7] "rs7267882" "rs8123328" "rs7265788" "rs2325971" "rs873711" "rs6037425"

> summary(gtdata(df5k[, directNotImp]))

      Chromosome Position Strand A1 A2 NoMeasured CallRate      Q.2
rs2104741         20   787070      +  G  A         120        1 0.004166667
rs7263171         20   855655      +  C  T         120        1 0.004166667
rs6110342         20  1458950      +  T  G         120        1 0.004166667
rs6105340         20  1493635      +  G  A         120        1 0.004166667
rs11905071        20  1557640      +  T  C         120        1 0.004166667
rs6080013         20  1590861      +  G  A         120        1 0.004166667
rs6074978         20  1595216      +  T  C         120        1 0.004166667
      P.11 P.12 P.22 Pexact      Fmax      Plrt
rs2104741  119    1    0      1 -0.0041841 0.948425
rs7263171  119    1    0      1 -0.0041841 0.948425
rs6110342  119    1    0      1 -0.0041841 0.948425
rs6105340  119    1    0      1 -0.0041841 0.948425
rs11905071 119    1    0      1 -0.0041841 0.948425
rs6080013  119    1    0      1 -0.0041841 0.948425
rs6074978  119    1    0      1 -0.0041841 0.948425

> mlinfo[which(mlinfo$SNP %in% directNotImp),]

      SNP A11 A12 Freq1      MAF Quality      Rsq
871  rs2104741  G  A 0.9562 0.0438 0.9145 0.0679
1027 rs7263171  C  T 0.8639 0.1361 0.9246 0.7898
1978 rs6110342  T  G 0.9160 0.0840 0.8472 0.1967
2028 rs6105340  G  A 0.8790 0.1210 0.7849 0.2076
2064 rs11905071 T  C 0.8846 0.1154 0.7989 0.1890
2111 rs6080013  G  A 0.9577 0.0423 0.9342 0.3869
2118 rs6074978  T  C 0.9576 0.0424 0.9340 0.3851

> summary(gtdata(df5k[, imputeNotDir]))

```

```

      Chromosome Position Strand A1 A2 NoMeasured CallRate      Q.2 P.11
rs553378          20   849882      +  G  A          120          1 0.02500000  114
rs6047425         20  2141014      +  A  G          120          1 0.07083333  104
rs6035871         20  2143364      +  A  G          120          1 0.07083333  104
rs6051434         20  2781237      +  T  G          120          1 0.47500000   37
rs6076466         20  2982048      +  T  C          120          1 0.22916667   71
rs2326056         20  2985607      +  C  A          120          1 0.22500000   72
      P.12 P.22      Pexact      Fmax      Plrt
rs553378          6      0 1.0000000 -0.025641026 0.6948707
rs6047425        15      1 0.4561335  0.050382485 0.6092978
rs6035871        15      1 0.4561335  0.050382485 0.6092978
rs6051434        52     31 0.1473878  0.131161236 0.1502658
rs6076466        43      6 1.0000000 -0.014250614 0.8754486
rs2326056        42      6 1.0000000 -0.003584229 0.9686478
> mlinfo[which(mlinfo$SNP %in% imputeNotDir),]
      SNP A11 A12  Freq1      MAF Quality      Rsq
1017 rs553378  G   A 0.9987 0.0013  0.9974 0.0005
2969 rs6047425 A   G 0.9388 0.0612  0.8964 0.2573
2973 rs6035871 A   G 0.9387 0.0613  0.8962 0.2582
3884 rs6051434 G   T 0.5186 0.4814  0.8427 0.7732
4027 rs6076466 T   C 0.7040 0.2960  0.6570 0.3365
4032 rs2326056 C   A 0.7338 0.2662  0.6422 0.2403
> summary(gtdata(df5k[,inBoth]))
      Chromosome Position Strand A1 A2 NoMeasured CallRate      Q.2 P.11
rs566570          20  2965113      +  T  C          120          1 0.495833333  33
rs6039167          20   846271      +  G  A          120          1 0.012500000  117
rs6037443          20  2909408      +  G  A          120          1 0.491666667   35
rs7261762          20   853448      +  A  G          120          1 0.008333333  118
rs554362           20   850002      +  A  G          120          1 0.012500000  117
rs7273309          20   853154      +  C  T          120          1 0.004166667  119
rs7267882          20   853785      +  G  A          120          1 0.004166667  119
rs8123328          20   855045      +  G  T          120          1 0.004166667  119
rs7265788          20   855426      +  A  G          120          1 0.004166667  119
rs2325971          20  2774477      +  T  C          120          1 0.470833333   38
rs873711           20  2775468      +  A  G          120          1 0.470833333   38
rs6037425          20  2785379      +  G  C          120          1 0.470833333   38
      P.12 P.22      Pexact      Fmax      Plrt
rs566570         55     32 0.3648758  0.083269672 0.3614010
rs6039167          3      0 1.0000000 -0.012658228 0.8454919
rs6037443         52     33 0.1467972  0.133092526 0.1442654
rs7261762          2      0 1.0000000 -0.008403361 0.8968501
rs554362           3      0 1.0000000 -0.012658228 0.8454919
rs7273309          1      0 1.0000000 -0.004184100 0.9484250
rs7267882          1      0 1.0000000 -0.004184100 0.9484250
rs8123328          1      0 1.0000000 -0.004184100 0.9484250
rs7265788          1      0 1.0000000 -0.004184100 0.9484250
rs2325971         51     31 0.1032427  0.147097763 0.1065638
rs873711          51     31 0.1032427  0.147097763 0.1065638
rs6037425         51     31 0.1032427  0.147097763 0.1065638

```

```
> mlinfo[which(mlinfo$SNP %in% inBoth),]
      SNP Al1 Al2 Freq1    MAF Quality    Rsq
1009 rs6039167  G   A 0.9875 0.0125  0.9995 0.9800
1018 rs554362   A   G 0.6496 0.3504  0.5748 0.2481
1022 rs7273309  C   T 0.9985 0.0015  0.9970 0.1896
1023 rs7261762  A   G 0.9913 0.0087  0.9991 0.9472
1024 rs7267882  G   A 0.9911 0.0089  0.9986 0.9230
1025 rs8123328  G   T 0.9902 0.0098  0.9964 0.8126
1026 rs7265788  A   G 0.9886 0.0114  0.9929 0.6884
3877 rs2325971  T   C 0.5811 0.4189  0.8526 0.7807
3880 rs873711   A   G 0.5888 0.4112  0.8597 0.7934
3886 rs6037425  G   C 0.5871 0.4129  0.8651 0.8034
3958 rs6037443  A   G 0.4975 0.4975  0.8918 0.8340
4009 rs566570   C   T 0.5307 0.4693  0.8814 0.8074
```

## Chapter 10

# Meta-analysis of GWA scans

### 10.1 Standard meta-analysis methods

Imagine you are interested in the effect of a certain polymorphism onto a particular disease. After scanning literature, you find some studies that implicate certain allele as significantly increasing the risk of the disease, but you will typically find also that other studies were inconclusive (no significant association), and that even some of the studies implicated the same allele as "protective". Your gut feeling may be that the allele is indeed the risk one, because you feel that the studies contradicting to this hypothesis were based on small number of subjects; however, how do you quantify this feeling? In this situation you need to perform meta-analysis of available data to come up with the joint effect size estimate and P-value, as based on all available data.

Let us first consider a situation when you are interested in the effect of the allele on a quantitative phenotype, expressed as a coefficient of regression of the trait onto the number of this allele in the genotype. Under a favorable scenario, from every individual study you would know the estimate of this regression coefficient, and the standard error of the estimate (or, equivalently, the *P-value* or the test statistics value for association).

One of approaches frequently used in meta-analysis of the data coming from a number of independent studies is the inverse variance method. In essence, this method is equivalent to combining likelihoods coming from separate studies, using quadratic approximation. Denote coefficients of regression estimated in  $N$  studies as  $\beta_i$ , and associated squared standard errors of the estimates as  $s_i^2$  where  $i \in 1, 2, \dots, N$ . Note that the regression coefficient should be reported on the same scale, e.g. centimeters, meters, or using observations reported on the standard normal scale. Define weights for individual studies as

$$w_i = \frac{1}{s_i^2}$$

Then the pooled estimate of the regression coefficient is

$$\beta = \frac{\sum_{i=1}^N w_i \beta_i}{\sum_{i=1}^N w_i}$$

As you can see, the weights have straightforward interpretation: the bigger the weight of the study (meaning the smaller is the standard error in the study), the larger is the contribution from this study onto the pooled estimate.

The standard error of the pooled estimate is computed as

$$s^2 = \frac{1}{\sum_{i=1}^N w_i}$$

and the  $\chi^2$ -test for association is computed in standard manner as

$$T^2 = \frac{\beta^2}{s^2} = \frac{\left(\sum_{i=1}^N w_i \beta_i\right)^2}{\sum_{i=1}^N w_i}$$

or, alternatively, the Z-test is

$$Z = \frac{\beta}{s} = \frac{\sum_{i=1}^N w_i \beta_i}{\sqrt{\sum_{i=1}^N w_i}}$$

Let us try to do meta-analysis using the inverse variance pooling method. Imagine we have information from four different studies reporting effect and the standard error of the same allele:

Table 10.1: Estimated regression coefficients from four studies

Study	$n$	$\beta$	$s_\beta$	$\chi^2$
1	225	0.16	0.07	5.224
2	560	0.091	0.042	4.694
3	437	0.072	0.048	2.25
4	89	-0.03	0.12	0.062
Total	1311	?	?	?

Let us try to access the joint significance of the association using these data. First, let us define a vector of regression coefficients and squared standard errors:

```
> beta <- c(0.16, 0.091, 0.072, -0.03)
> s <- c(0.07, 0.042, 0.048, 0.12)
> s2 <- s*s
> s2

[1] 0.004900 0.001764 0.002304 0.014400
```

Compute the weight for individual studies as

```
> w <- 1/s2
> w

[1] 204.08163 566.89342 434.02778 69.44444
```

Estimate pooled regression coefficient as

```
> pbeta <- sum(w*beta)/sum(w)
> pbeta
```



```
[1] 0.08898527
```

and pooled squared standard error as

```
> ps2 <- 1/sum(w)
> ps2
```

```
[1] 0.0007846539
```

To access significance of association in meta-analysis, let us compute  $\chi^2$  test statistics and the  $P$  – value with

```
> pchi2 <- pbeta*pbeta/ps2
> pchi2
```

```
[1] 10.09155
```

```
> ppvalue <- 1. - pchisq(pchi2,1)
> ppvalue
```

```
[1] 0.001489504
```

We conclude that there is a significant association in meta-analysis.

There is an important effect which should be considered when doing meta-analysis of published data. Given numerous polymorphisms available in human genome, a particular polymorphism usually becomes a focus of interest only when it was shown to be significantly associated in some study which reports it. Put it other way around: only when a significant association was detected and reported, more studies are likely to be performed on the same polymorphism. This first report, however, is not guaranteed to demonstrate a true association: it may well report a false-positive, or, even in presence of association, it is likely to over-estimate the effect of the polymorphism. Thus there is a positive bias in literature reports, and this bias is particularly strong for the first report, a phenomenon frequently referenced to as "champion's" or "winner's curse".

The observations we have just considered are quite typical in that the first study, where the association was originally discovered, reports the biggest effect and most significant effect, while the follow-up studies suggest smaller effect.

Therefore, when you meta-analyse data from publications it is always good idea to exclude the first report (in case it is positive – and it is always positive!) and check if significant association is still observed. Let us try to do that:

```
> beta <- beta[2:4]
> s2 <- s2[2:4]
> w <- w[2:4]
> pbeta <- sum(w*beta)/sum(w)
> pbeta
```

```
[1] 0.07544522
```

```
> ps2 <- 1/sum(w)
> ps2
```

```
[1] 0.0009342602
```

```

> pchi2 <- pbeta*pbeta/ps2
> pchi2

[1] 6.092501

> ppvalue <- 1. - pchisq(pchi2,1)
> ppvalue

[1] 0.01357568

```

Indeed, when the first "champion" report is excluded, the overall evidence is decreased and results become less significant, though still pointing to the same direction.

When binary traits are studied, and results are reported as Odds Ratios with  $P$ -values, it is also possible to apply inverse variance method. For this, you need to transform your Odds Ratios using natural logarithm, and, on this scale, estimate the standard error. Generic inverse variance pooling may be applied to the data transformed this way; the final results are back-transformed onto Odds Ratio scale using exponentiation.

Let us consider a simple example. Let Odds Ratios and  $\chi^2$  test statistics values coming from four studies of a binary phenotype are as following:  $\theta_1 = 1.5$  ( $\chi^2 = 5.1$ ),  $\theta_2 = 1.3$  ( $\chi^2 = 2.2$ ),  $\theta_3 = 0.9$  ( $\chi^2 = 0.5$ ),  $\theta_4 = 1.2$  ( $\chi^2 = 3.1$ ).

Let us first transform the Odds Ratio to the logarithmic scale with

```

> or <- c(1.5,1.3,0.9,1.2)
> lnor <- log(or)
> lnor

[1] 0.4054651 0.2623643 -0.1053605 0.1823216

```

To compute standard errors from known  $\chi^2$  values, one can use simple relation

$$\chi^2 = \frac{\beta^2}{s^2}$$

and thus

$$s^2 = \frac{\beta^2}{\chi^2}$$

Thus to compute the square standard errors corresponding to the log-Odds Ratio, we can use

```

> chi2or <- c(5.1,2.2,0.5,3.1)
> s2lnor <- lnor*lnor/chi2or
> s2lnor

[1] 0.03223568 0.03128864 0.02220168 0.01072295

```

Now we can combine log-Odds Ratios and corresponding standard errors using inverse variance method:

```

> w <- 1/s2lnor
> plnor <- sum(w*lnor)/sum(w)
> plnor

```

```
[1] 0.1650462
> ps2 <- 1/sum(w)
> ps2
[1] 0.004968165
> pchi2 <- plnor*plnor/ps2
> pchi2
[1] 5.482958
> ppval <- 1.-pchisq(pchi2,1)
> ppval
[1] 0.01920274
```

And the corresponding estimate of pooled Odds Ratio is

```
> exp(plnor)
[1] 1.179448
and 95% confidence interval is
> exp(plnor-1.96*sqrt(ps2))
[1] 1.02726
> exp(plnor+1.96*sqrt(ps2))
[1] 1.354181
```

Some times, effects are reported on different scales, and/or there may be suspect that these effects are not translatable across studies because of the differences in experimental design or for some other reasons. In this case, it may be better to pool the data without use of the effect estimate in exact manner, based only on the sign of association and its significance. This can be done by pooling Z-score values. Z-score refers to the test statistics, which has standard normal distribution under the null and can be derived e.g. by dividing estimate of the regression coefficient onto its standard error:

$$Z_i = \frac{\beta_i}{s_i}$$

The Z-score pooling methods can be derived from the inverse variance pooling by exploiting the fact that generally standard error of the estimate is proportional to  $1/\sqrt{n}$ , where  $n$  is the number of observations used for estimation. Therefore individual scores are assigned weights which are proportional to the square root of number of independent observations used in individual study,  $w_i = \sqrt{n_i}$ . The pooled Z-score statistics is computed as

$$Z = \frac{\sum_{i=0}^N w_i Z_i}{\sqrt{\sum_{i=0}^N w_i^2}}$$

We can now repeat the analysis of our first data set using Z-score pooling method. First, our data from table 10.1 are

```
> n <- c(225,560,437,89)
> beta <- c(0.16,0.091,0.072,-0.03)
> s <- c(0.07,0.042,0.048,0.12)
```

The Z-scores and weights are are:

```
> z <- beta/s
> z

[1] 2.285714 2.166667 1.500000 -0.250000

> w <- sqrt(n)
> w

[1] 15.000000 23.664319 20.904545 9.433981
```

The pooled estimate of Z-score is

```
> pz <- sum(w*z)/sqrt(sum(w^2))
> pz

[1] 3.163875
```

and corresponding  $P$  – value is

```
> 1.-pchisq(pz*pz,1)

[1] 0.001556839
```

which is almost the same  $P$  – value we have obtained previously using the inverse variance method. Note, however, that now we do not know the “pooled” estimate of the regression coefficient.

Other important aspects of meta-analysis, such as heterogeneity, and a wide range of methods different from the inverse variance and Z-score based methods are not covered here, and we refer the reader to more epidemiologically-oriented literature for a better review.

## 10.2 Exercise: meta-analysis of literature data

In this exercise, you will perform meta-analysis of results collected from literature. These results resemble those obtained for association analysis between Pro12Ala polymorphism of the PPAR-GenABEL-package *emma* gene and type 2 diabetes. The data collected from literature are presented in the table 10.2.

As you can see, only the original study report significant association, while other four are insignificant and one point in opposite direction.

Answer the following questions:

**Exercise 8** *Perform meta-analysis of the data presented in table 10.2. Which allele is the risk one? Is this risk significant? What is pooled Odds Ratio and 95% confidence interval? Do analysis using at least two methods. Which method is better (best) in this situation? Why?*

**Exercise 9** *Perform meta-analysis excluding the original report (study 1). Is there still significant association between Pro12Ala and diabetes?*

Table 10.2: Summary of six studies of association between T2D and Pro12Ala polymorphism of the PPAR-GenABEL-`packagemma` gene.  $n$ : number of subjects; effective allele: the allele for which the OR was estimated.

Study	Effective allele	$n$	$OR_E$	$P - value$
1	Ala	221	0.67	0.013
2	Pro	306	0.93	0.60
3	Pro	71	1.08	0.84
4	Ala	164	0.83	0.40
5	Pro	242	1.22	0.25
6	Pro	471	1.23	0.07

### 10.3 Reporting GWA results for future meta-analysis

In this section, we will discuss specifics of GWA analysis when meta-analysis is aimed at later stage. In order to perform meta-analysis at later stage, using either inverse variance or Z-score based method, you generally need to report only effect estimates, standard errors of The estimates (or, equivalently,  $P - values$  or test statistics values), and number of observations used for estimation.

It is also clear that it is crucial to know for which allele the effect is reported, and this is the point where meta-analysis of genetic data may be very confusing. Generally, one may think that reporting what couple of nucleotide bases correspond to the polymorphism under the study and defining what allele was used as reference in the regression model may be enough. This, however, is not true for certain class of polymorphisms and may be a source of great confusion.

Consider a DNA molecule; as you know it is made of two complementary strands (forward or "+" and reverse or "-"). As you may guess, depending on the strand, what is an "A/G" polymorphism when reported on "+" strand becomes "T/C" polymorphism on the "-" strand (using complementarity rule A<->T and G<->C). This is not a big problem for most of the polymorphism classes, because if say you know that for a first study  $\beta_1$  is reported for the "G" allele of the "A/G" polymorphism and in the second study  $\beta_2$  is the estimate of the effect "C" allele of the same polymorphism, but coded as "T/C" (thus other strand), you can easily spot that and say the "C" is the same as "G" in this situation, and pool the two betas straightforwardly.

However, for two types of polymorphisms, "A/T" and "G/C", where you can not get away without knowing what the strand was: what is reported as the effect of "T" in "A/T" polymorphism in study one; and seemingly corresponding effect of "T" in "A/T" polymorphism in study two may be apparently reports for two opposite alleles, if strands used for reporting in two studies were different.

The story may become even more complex, because the forward/reverse orientation depends on the genomic build<sup>1</sup>.

Thus if you want to pool your results with the results of others, there are quite a few SNP characteristics which are absolutely crucial to report, namely, the nucleotide bases describing the polymorphism, with indication which one was used as reference, and which one as "effective", strand, genomic build, and only

<sup>1</sup> There is alternative, top/bottom, strand designation, which does not depend on genomic build. However, it is not always used.

than the effect estimate, standard error of the effect, and number of observations used to do estimation.

Other characteristics which are also recommended for reporting because they describe the quality characteristics of the sample and/or provide redundant information, which is good for double checks. Such characteristics include: frequency of the effective or reference allele, call rate, P-value for Hardy-Weinberg equilibrium and may be some parameter describing what is the direction of deviation from HWE (e.g.  $F_{max}$ ). When reporting results for imputed SNPs, more quality control characteristics should be included, such as average maximal posterior probability,  $R^2$ , etc.

Let us start with arranging two data sets which could then be used for meta-analysis. Basically, we will use cleaned data from the GWA exercise you did in section 5 ("Genome-wide association analysis", page 101), and split that in two parts.

If you did not do this yet, start R and load **GenABEL-package** library, which you will need it to work with GWA-data

```
> library(GenABEL)
```

Load the data with

```
> load("data2.RData")
```

and then split it in two parts:

```
> nids(data2)
```

```
[1] 124
```

```
> mdt1 <- data2[1:40,]
```

```
> mdt2 <- data2[41:nids(data2),]
```

We will analyse body mass index. If you pooling results of analysis of studies which are designed in approximately the same manner, you may think of reporting the effect estimates on the same scale and use of the inverse variance method for meta-analysis.

However, in meta-analysis of multiple data sets different individual studies are likely to assess different population, will use different designs, measure different covariates, and so on. Therefore you should think of some standardisation of the outcome variable (or apply Z-score method).

Therefore for the purpose of future meta-analysis, it becomes conventional to analyse pre-adjusted data which are scaled to Standard Normal (mean of zero and variance of unity). Note that this argument applies only to meta-analysis – you may and should report effects on the original scale (e.g. in centimeters and grams) in analysis of individual studies, in order to have better interpretability.

Moreover, in meta-analysis you heavily rely on the large numbers approximation when estimating  $P$ -values; while for individual study you can always apply empirical, e.g. permutation-based, procedures to derive the correct  $P$ -value whatever is the distribution of the trait, in meta-analysis the Normality assumption becomes crucial, and you do not want few outliers spoiling your results by screwing up  $P$ -values. Therefore some transformation improving normality is

desirable. Note that transformation to Standard Normal does not improve the fit to normality; to do that other transformation should be applied. Probably the most famous transformations are log- and square root ones, then one may think of Box-Cox transformation. At the same time there is a transformation, called a Rank Transformation to Normality which guarantees perfect fit to Normal in absence of heavy ties<sup>2</sup>. We advocate the use of Rank Transformation to Normal for meta-analysis purposes.

**GenABEL**-package implements the `ztransform` function for the purposes of Z-transformation. This function takes two (actually three – see help for details) arguments: formula (or just the variable name) and data. `ztransform` function will perform (generalised) linear regression using the specified formula, and will transform the residuals from analysis onto Z-scale by subtracting the mean and division by the standard deviation.

Let us consider what this function does practically. Let us first transform BMI from the first set without using covariates:

```
> zBMI0 <- ztransform(bmi,mdta1)
```

The histogram of the transformed variable and scatter-plot of raw against transformed BMI is given at figure 10.1, column 1. Note that the fit to Normality is not improved by this transformation; with the original BMI, the Shapiro test for deviation from normality gives

```
> shapiro.test(phdata(mdta1)$bmi)
```

Shapiro-Wilk normality test

```
data:  phdata(mdta1)$bmi
W = 0.9328, p-value = 0.0199
```

with identical results from the transformed variable:

```
> shapiro.test(zBMI0)
```

Shapiro-Wilk normality test

```
data:  zBMI0
W = 0.9328, p-value = 0.0199
```

This is quite natural: as you can note from scatter-plot in column 1 of figure 10.1, only the centering and the spread of the scales are different for X (original BMI) and Y ( $x_0$ ), otherwise there is an exact linear correspondence between the two.

We can also do transformation using sex and age-adjusted residuals with

```
> zBMI1 <- ztransform(bmi~sex+age,mdta1)
```

The scatter-plot of raw against transformed BMI is given at figure 10.1, column 2. Note that this transformation may slightly change the fit to Normal, which happens because we factor out the effects of sex and age:

```
> shapiro.test(zBMI1)
```

---

<sup>2</sup> Ties are generated by the subjects with exactly the same trait values

## Shapiro-Wilk normality test

```
data:  zbmi1
W = 0.9263, p-value = 0.01224
```

From the scatter-plot in column 2 of figure 10.1, it is quite clear what happens: the residuals from linear regression are not corresponding to the original BMI in exact linear manner.

A similar function, which performs rank-transformation to normality, is named `rntransform`. For example if we want to adjust BMI for sex and age and rank-transform the residuals to Normal, we can use

```
> rnbmi1 <- rntransform(bmi~sex+age,mdta1)
```

This transformation, however, indeed improves the fit to Normal:

```
> shapiro.test(rnbmi1)
```

## Shapiro-Wilk normality test

```
data:  rnbmi1
W = 0.999, p-value = 1
```

In essence, the  $P$  – value of 1 means perfect fit to Normal – and this is what should have occurred when this transformation is used on the data without ties. Perfectly Normal distribution of the transformed trait may be enjoyed at the histogram presented at column 3 of figure 10.1.

Let us analyse Rank-Normal-transformed, sex and age-adjusted BMI in the two data sets, using `qtscore` function. Analysis of the first study is done with

```
> qts1 <- qtscore(rnbmi1,mdta1)
```

and analysis of the second study is done with

```
> zbmi2 <- ztransform(bmi~sex+age,mdta2)
> qts2 <- qtscore(zbmi2,mdta2)
```

The analysis looks very simple – is not it? However, the real difficulty did not start yet: now we need to extract coding, reference allele, strand, etc. – otherwise we can not do right meta-analysis later on!

Let us assume that you want to summarise the GW results from additive 1 d.f. test using following variables (as, e.g., requested by consortium):

- **name:** SNP name
- **chromosome:** chromosome number
- **position:** physical position of the SNP
- **refallele:** reference allele
- **codedallele:** coded (effect) allele
- **strand:** strand
- **refallfreq:** frequency of the reference allele



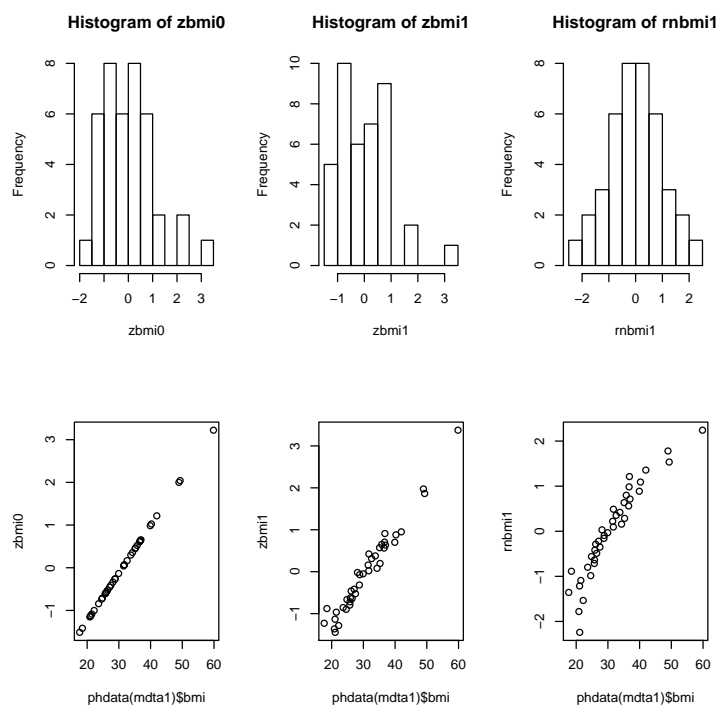


Figure 10.1: Histogram of transformed BMI and scatter-plots of the raw BMI against transformed BMI. Column 1: Z-transformation without covariates. Column 2: Z-transformation with adjustment for age and sex. Column 3: Rank-transformation to normality, after adjustment for sex and age.

- **n**: number of people with data available for this SNP test
- **beta**: estimate of the effect of the allele
- **se.beta**: standard error of the effect estimate
- **p**: P-value for the test
- **p\_corr**: corrected P-value (we will use Genomic Control)
- **call**: SNP call rate
- **phwe**: *P*-value from the exact test for HWE

Let us look what we get as an output from `qtscore` analysis:

```
> results(qts1)[1:2,]
```

	Chromosome	Position	Strand	A1	A2	N	effB	se_effB	chi2.1df
rs1646456	1	653	+	C	G	40	0.1768955	0.2560635	0.4772414
rs4435802	1	5291	+	C	A	40	-0.4064900	0.4095768	0.9849838
	P1df	effAB	effBB	chi2.2df	P2df	Pc1df			
rs1646456	0.4896745	0.09703712	0.4909062	0.6323627	0.7289272	0.4896745			
rs4435802	0.3209715	-0.40649004		NA	0.9849838	0.3209715	0.3209715		

You can see that most information is already present in the output, though called using names which are different from these requested. However, we miss reference allele frequency, SNP call rate and *P*-value from the Hardy-Weinberg equilibrium test. These however can be computed using the `summary` function:

```
> # for data part 1:
> sum1 <- summary(gtdata(mdt1))
> sum1[1:2,]
```

	Chromosome	Position	Strand	A1	A2	NoMeasured	CallRate	Q.2	P.11	P.12
rs1646456	1	653	+	C	G	40	1	0.3375	16	21
rs4435802	1	5291	+	C	A	40	1	0.0875	33	7

```
P.22 Pexact Fmax Plrt
rs1646456 3 0.4775382 -0.17400419 0.2616425
rs4435802 0 1.0000000 -0.09589041 0.4122629
```

```
> # ... and for data part 2:
> sum2 <- summary(gtdata(mdt2))
> sum2[1:2,]
```

	Chromosome	Position	Strand	A1	A2	NoMeasured	CallRate	Q.2	P.11
rs1646456	1	653	+	C	G	83	0.9880952	0.33734940	36
rs4435802	1	5291	+	C	A	82	0.9761905	0.07926829	69

```
P.12 P.22 Pexact Fmax Plrt
rs1646456 38 9 1 -0.02402597 0.826397
rs4435802 13 0 1 -0.08609272 0.289791
```

Note, however, that we now got frequency of the *effective* (or coded) allele, not the frequency of the reference allele! The quantity we need can be easily computed, though:

```
> # for data part 1
> refallfreq1 <- 1 - sum1[,"Q.2"]
> # ... and for data part 2
> refallfreq2 <- 1 - sum2[,"Q.2"]
```

At this moment we can arrange the required data frame:

```
> mdf1 <- data.frame(name=snpnames(qts1), chromosome=chromosome(qts1),
+ position=map(qts1), refallele=refallele(qts1),
+ codedallele=effallele(qts1), strand = strand(qts1),
+ refallelefreq = refallfreq1, n=qts1[, "N"],
+ beta=qts1[, "effB"],
+ se_beta=qts1[, "se_effB"], p=qts1[, "P1df"],
+ p_corr=qts1[, "Pc1df"], call = sum1[, "CallRate"],
+ phwe = sum1[, "Pexact"], stringsAsFactors = FALSE)
```

note the last argument – `stringsAsFactors = FALSE`. I suggest you use that by default when constructing a new data frame – unless you are sure that you can work out your way later on with strings saved as factors.

Let us inspect the first 5 rows of the resulting output:

```
> mdf1[1:5,]
```

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	name	chromosome	position	refallele	codedallele	strand	
rs1646456	rs1646456	1	653	C	G	+	
rs4435802	rs4435802	1	5291	C	A	+	
rs946364	rs946364	1	8533	T	C	-	
rs299251	rs299251	1	10737	A	G	+	
rs2456488	rs2456488	1	11779	G	C	+	

	refallele	freq	n	beta	se_beta	p	p_corr	call
rs1646456	0.6625000	40	0.1768955	0.2560635	0.4896745	0.4896745	1.000	
rs4435802	0.9125000	40	-0.4064900	0.4095768	0.3209715	0.3209715	1.000	
rs946364	0.7236842	38	0.2944870	0.2752734	0.2847102	0.2847102	0.950	
rs299251	0.9615385	39	0.4958353	0.5989959	0.4077965	0.4077965	0.975	
rs2456488	0.6625000	40	0.1145684	0.2271545	0.6140062	0.6140062	1.000	

phwe

rs1646456	0.4775382
rs4435802	1.0000000
rs946364	0.6911168
rs299251	1.0000000
rs2456488	0.7320709

However, it is not recommended that you perform above-described reporting actions unless you develop your own format. In case if you plan to use **MetABEL-package** for meta-analysis, you best use **formetascore** function, which basically performs operations similar to described, and reports results in format compatible with **MetABEL-package**.

Thus, if you plan to use **MetABEL-package** for meta-analysis, required tables can be generated with single command:

```
> mdf1 <- formetascore(bmi~sex+age,mdta1,transform=rntransform, verbosity = 2 )
```

You can see that results are the same as previously:

```
> mdf1[1:5,]
```

	name	chromosome	position	strand	allele1	allele2	build
rs1646456	rs1646456	1	653	+	C	G	unknown
rs4435802	rs4435802	1	5291	+	C	A	unknown
rs946364	rs946364	1	8533	-	T	C	unknown
rs299251	rs299251	1	10737	+	A	G	unknown
rs2456488	rs2456488	1	11779	+	G	C	unknown

	effallele	effallelefreq	n	beta	sebeta	p	pgc
rs1646456	G	0.33750000	40	0.1768955	0.2560635	0.4896745	0.4896745
rs4435802	A	0.08750000	40	-0.4064900	0.4095768	0.3209715	0.3209715
rs946364	C	0.27631579	38	0.2944870	0.2752734	0.2847102	0.2847102
rs299251	G	0.03846154	39	0.4958353	0.5989959	0.4077965	0.4077965
rs2456488	C	0.33750000	40	0.1145684	0.2271545	0.6140062	0.6140062

	lambda.estimate	lambda.se	lambda.iz0	lambda.iz2	pexhwe	call
rs1646456	1	NA	1.006539	1	0.4775382	1.000
rs4435802	1	NA	1.006539	1	1.0000000	1.000
rs946364	1	NA	1.006539	1	0.6911168	0.950
rs299251	1	NA	1.006539	1	1.0000000	0.975
rs2456488	1	NA	1.006539	1	0.7320709	1.000

To write all the data to a file, we can use standard R `write.csv` function:

```
> write.csv(mdf1, file="RData/part1.rnbmisexage.csv", row.names=F)
```

Similar analysis is applied to the second data set:

```
> mdf2 <- formetascore(bmi~sex+age,mdta2,transform=rntransform, verbosity = 2 )
```

We can inspect the first five lines of the output with

```
> mdf2[1:5,]
```

	name	chromosome	position	strand	allele1	allele2	build
rs1646456	rs1646456	1	653	+	C	G	unknown
rs4435802	rs4435802	1	5291	+	C	A	unknown
rs946364	rs946364	1	8533	-	T	C	unknown
rs299251	rs299251	1	10737	+	A	G	unknown
rs2456488	rs2456488	1	11779	+	G	C	unknown

	effallele	effallelefreq	n	beta	sebeta	p
rs1646456	G	0.32926829	82	-0.04879397	0.1663650	0.76929696
rs4435802	A	0.08024691	81	0.37724197	0.2984226	0.20618701
rs946364	C	0.25903614	83	-0.14414880	0.1790329	0.42073156
rs299251	G	0.04216867	83	-0.69920378	0.3919648	0.07444912
rs2456488	C	0.34146341	82	-0.23105805	0.1520352	0.12856957

	pgc	lambda.estimate	lambda.se	lambda.iz0	lambda.iz2
rs1646456	0.77676571	1.070017	0.0009650323	1.036357	1.027149
rs4435802	0.22168453	1.070017	0.0009650323	1.036357	1.027149
rs946364	0.43635427	1.070017	0.0009650323	1.036357	1.027149
rs299251	0.08461892	1.070017	0.0009650323	1.036357	1.027149
rs2456488	0.14177789	1.070017	0.0009650323	1.036357	1.027149

	pexhwe	call
rs1646456	0.8038996	0.9879518
rs4435802	1.0000000	0.9759036
rs946364	1.0000000	1.0000000
rs299251	1.0000000	1.0000000
rs2456488	0.4685397	0.9879518

Let us write the data to a file:

```
> write.csv(mdf2, file="RData/part2.rnbmisexage.csv", row.names=F)
```

Finally let us analyse and save results for another data set, `ge03d2c`:

```
> data(ge03d2c)
```

```
> mdf3 <- formetascore(bmi~sex+age,ge03d2c,transform=rntransform, verbosity = 2 )
```

```
> write.csv(mdf3, file="RData/part3.rnbmisexage.csv", row.names=F)
```

## 10.4 Meta-analysis with MetABEL-package

Now we are ready to meta-analyse GWA data coming from three studies. For this we will need to use `MetABEL-package` package, implementing simple meta-analysis functions for GWA data. Start with loading the package:

```
> library(MetABEL)
```

We will first meta-analyse the three studies using the data frames generated in previous section, `mdf1`, `mdf2` and `mdf3`. For this we will use the core function of `MetABEL`-package, `metagwa.tables`. This function takes four arguments: two data frames with results from individual studies, and two arguments supplying the study names. Pooling of multiple studies is possible by sequential application of this function.

Let us pool two first data frames:

```
> pooled <- metagwa.tables(mdf1,mdf2,name.x="Part1",name.y="Part2")
```

```
analysing ...
```

```
Lambda Part1 = 0.9499903
```

```
Lambda Part2 = 1.098705
```

```
Corrected Lambda Part1 = 0.9499903
```

```
Corrected Lambda Part2 = 1
```

```
Lambda POOLED data = 1.023276
```

```
... DONE
```

The pooled data frame contains results of meta-analysis and essential details of the original studies:

```
> pooled[1:5,]
```

	name	strand	allele1	allele2	effallele	chromosome	position	n	npops			
1	rs100616	-	G	C	C	1	1911712	122	2			
2	rs1006497	+	T	G	G	1	2658810	122	2			
3	rs1011580	+	A	G	G	3	10048771	121	2			
4	rs1011953	+	A	G	G	2	6464510	123	2			
5	rs1013473	+	A	T	T	1	4487262	123	2			
	beta	sebeta	effallelefreq	call	pexhwe	obetaPart1						
1	0.11443814	0.1850338	0.1270492	0.9919020	4.64447636	0.08819818						
2	-0.07946221	0.1954998	0.1434426	0.9920082	0.16813927	-0.25425751						
3	-0.06801448	0.1397522	0.5041322	0.9837773	3.15056880	-0.06755996						
4	0.07539928	0.1372443	0.3292683	1.0000000	0.10373542	0.18982068						
5	0.30393338	0.1334451	0.5121951	1.0000000	0.04821391	0.31522388						
	obetaPart2	osePart1	osePart2	chi2	p							
1	0.1230224623	0.3726827	0.2131624	0.3825070	0.5362646							
2	-0.0001117686	0.3498751	0.2357343	0.1652071	0.6844070							
3	-0.0683575796	0.2130800	0.1851327	0.2368565	0.6264858							
4	0.0055254619	0.2228918	0.1741797	0.3018185	0.5827446							
5	0.2978861467	0.2259542	0.1653644	5.1874252	0.0227509							

If one needs to pool more studies, this data frame should be used as the first argument of the `metagwa.tables`, and `name.x` argument should take special value "POOLED":

```
> pooled <- metagwa.tables(pooled,mdf3,name.x="POOLED",name.y="mdf3")
```

```
NA for betas in both populaions
```

```
18 SNPs removed
```

```

analysing ...
Lambda mdf3 = 1.128096
Corrected Lambda mdf3 = 1
Lambda POOLED data = 1.340905
... DONE

> pooled[1:5,]
      name strand allele1 allele2 effallele chromosome position  n npops
1 rs1000475      +      T      C      C          X 13721802  91    1
2 rs1000909      -      A      G      G          2  8531681 190    1
3 rs1006092      -      T      G      G          X 13527448 190    1
4  rs100616      -      G      C      C          1 1911712 310    3
5 rs1006497      +      T      G      G          1 2658810 315    3
      beta      sebeta effallelefreq      call      pexhwe      obetaPart1
1 -0.05521349 0.11185255      0.5824176 0.4690722 5.9671619      NA
2 -0.08504871 0.13218866      0.8157895 0.9793814 2.8521518      NA
3 -0.03712065 0.08523891      0.5078947 0.9793814 6.8378385      NA
4  0.11824774 0.12085884      0.1241935 0.9780568 6.5922479 0.08819818
5 -0.02366946 0.11439872      0.1603175 0.9937465 0.2313494 -0.25425751
      obetaPart2      obetamdf3      osePart1      osePart2      osemdf3      chi2
1      NA -0.055213494      NA      NA 0.11185255 0.24366808
2      NA -0.085048708      NA      NA 0.13218866 0.41394922
3      NA -0.037120652      NA      NA 0.08523891 0.18965112
4 0.1230224623 0.121082405 0.3726827 0.2131624 0.15961076 0.95725763
5 -0.0001117686 0.005382408 0.3498751 0.2357343 0.14107317 0.04280893
      p
1 0.6215693
2 0.5199718
3 0.6632071
4 0.3278788
5 0.8360855

```

This procedure may become quite laborious if multiple studies are to be pooled. In this case, it is possible to run meta-analysis using data provided in files, by applying function `metagwa.files`. As the first argument, this function takes the path to the directory where the files with results of individual studies are stored. It is assumed that the file names are made of two parts: population/study name and an extension. Thus the second argument of the `metagwa.files` function is the vector with names of studies, and the third one provides extension. Other arguments, "maf", "call" and "phwe" provide the threshold for QC filtering of SNPs in individual studies.

The function does not return any value, but rather creates a new file named `POOLEDextens`, where "extens" is the argument supplied to the function, in the source directory. To run analysis on the three files in the directory "RData" we can use

```

> metagwa.files(dir="RData",pops=c("part1","part2","part3"),extens=".rnbmisexage.csv")

Population part1 , reading RData/part1.rnbmisexage.csv done
Dimesions after filters are 3482 20

```

```

population part2, reading RData/part2.rnbmisexage.csv done
Dimesions after filters are 3530 20
analysing ...
Lambda part1 = 0.9493588
Lambda part2 = 1.095788
Corrected Lambda part1 = 0.9493588
Corrected Lambda part2 = 1
Lambda POOLED data = 1.024196
... DONE
Dimesions after pooling are 3533 20
population part3, reading RData/part3.rnbmisexage.csv done
Dimesions after filters are 7444 20
analysing ...
Lambda part3 = 1.12461
Corrected Lambda part3 = 1
Lambda POOLED data = 1.345936
... DONE
Dimesions after pooling are 7444 22
$analysed.pops
[1] "part1" "part2" "part3"

```

extra arguments regulate the SNP exclusion criteria: `maf=0.01` tells to exclude SNPs with minor allele frequency less then 0.5%, `call=0.95` tells to drop SNPs with call rate less than 95%, and `phwe=1.e-8` instructs to exclude SNPs with HWE  $P$ -value  $\leq 10^{-8}$ .

Now we can read and inspect the results of meta-analysis with:

```

> poolf <- read.csv("RData/POOLED.rnbmisexage.csv",strings=F)
> poolf[1:5,]

```

	name	strand	allele1	allele2	effallele	chromosome	position	n	npops
1	rs1000909	-	A	G	G	2	8531681	190	1
2	rs1006092	-	T	G	G	X	13527448	190	1
3	rs100616	-	G	C	C	1	1911712	310	3
4	rs1006497	+	T	G	G	1	2658810	315	3
5	rs1010481	+	A	C	C	2	8409087	190	1

	beta	sebeta	effallelefreq	call	pexhwe	obetapart1
1	-0.08504871	0.13198426	0.8157895	0.9793814	2.8521518	NA
2	-0.03712065	0.08510711	0.5078947	0.9793814	6.8378385	NA
3	0.11825684	0.12070003	0.1241935	0.9780568	6.5922479	0.08819818
4	-0.02359567	0.11424656	0.1603175	0.9937465	0.2313494	-0.25425751
5	-0.03241475	0.12358721	0.2657895	0.9793814	0.0000000	NA

	obetapart2	obetapart3	osepart1	osepart2	osepart3	chi2
1	NA	-0.085048708	NA	NA	0.13198426	0.41523235
2	NA	-0.037120652	NA	NA	0.08510711	0.19023899
3	0.1230224623	0.121082405	0.3726827	0.2128792	0.15936396	0.95992605
4	-0.0001117686	0.005382408	0.3498751	0.2354211	0.14085503	0.04265582
5	NA	-0.032414750	NA	NA	0.12358721	0.06879205

	p
1	0.5193256
2	0.6627178

```
3 0.3272055
4 0.8363747
5 0.7931037
```

## 10.5 Answers to the exercise

Perform meta-analysis of the data presented in table 10.2. Which allele is the risk one? Is this risk significant? What is pooled Odds Ratio and 95% confidence interval? Do analysis using at least two methods. Which method is better (best) in this situation? Why?

We first need to unify Odds Ratios by using the same effective allele. Let that be the "risk" allele, as may be guessed from a glance to the data, namely "Pro". When the effects are reported for the other, "Ala" allele, the corresponding ORs for the "Pro" allele can be found using simple relation  $OR_{Pro} = 1/OR_{Ala}$ .

Thus, the vector of Odds Ratios for "Pro" allele is

```
> or.pro <- c(1./0.67,0.93,1.08,1./0.83,1.22,1.23)
> or.pro

[1] 1.492537 0.930000 1.080000 1.204819 1.220000 1.230000
```

The corresponding  $P$  - values are

```
> p <- c(0.013,0.6,0.84,0.40,0.25,0.07)
```

Let us find log-ORs

```
> logor.pro <- log(or.pro)
> logor.pro

[1] 0.40047757 -0.07257069 0.07696104 0.18632958 0.19885086 0.20701417
```

Corresponding squared standard errors are

```
> s2 <- logor.pro*logor.pro/qchisq(1-p,1)
> s2

[1] 0.02599764 0.01915121 0.14531060 0.04901514 0.02988102 0.01305349
```

and weights are

```
> w <- 1/s2
> w

[1] 38.465034 52.216009 6.881811 20.401860 33.466060 76.607876
```

Thus the pooled estimate of log-OR is

```
> p.logor.pro <- sum(w*logor.pro)/sum(w)
> p.logor.pro

[1] 0.1686548
```

and the standard error is



```
> p.s <- 1/sqrt(sum(w))
> p.s
```

```
[1] 0.06622101
```

Thus the pooled estimate of Odds Ratio from association between type 2 diabetes and "Ala" allele is

```
> exp(p.logor.pro)
```

```
[1] 1.183711
```

and the 95% confidence interval is

```
> exp(p.logor.pro-1.96*p.s)
```

```
[1] 1.039627
```

```
> exp(p.logor.pro+1.96*p.s)
```

```
[1] 1.347765
```

The  $\chi^2$  test for association and corresponding  $P$  – value are

```
> p.chi2 <- (p.logor.pro/p.s)^2
> p.chi2
```

```
[1] 6.486429
```

```
> p.pval <- 1-pchisq(p.chi2,1)
```

```
> p.pval
```

```
[1] 0.01087011
```

Z-score pooling though may be more appropriate method for such differentially designed studies (e.g. control groups are very different). To get Z-score pooling working, we need first find Z-scores from P-values

```
> p <- c(0.013,0.6,0.84,0.40,0.25,0.07)
> z <- sqrt(qchisq(1-p,1))
> z
```

```
[1] 2.4837693 0.5244005 0.2018935 0.8416212 1.1503494 1.8119107
```

and assign the right sign (let "+" is for the risk effect of "Pro").

```
> effsig <- c(1,-1,1,1,1,1)
> z <- z*effsig
> z
```

```
[1] 2.4837693 -0.5244005 0.2018935 0.8416212 1.1503494 1.8119107
```

Now, we need to assign weights to the studies as

```
> n <- c(221,306,71,164,242,471)
> w <- sqrt(n)
```

ane the pooled estimate of  $Z$  and corresponding  $P$  – value are

```
> zpoo <- sum(w*z)/sqrt(sum(w^2))
> zpoo

[1] 2.537333

> 1-pchisq(zpoo*zpoo,1)

[1] 0.01117008
```

As you can see the results are almost identical to the previous obtained with inverse variance pooling.

### 10.5.1 Exercise 9:

Perform meta-analys excluding the original report (study 1). Is there still significant association between Pro12Ala and diabetes?

The answer to this exercise can be obtained in exactly the same manner, as for the previous one, limiting our consideration to the last five studies.

Thus, the vector of Odds Ratios for "Pro" allele is

```
> or.pro <- c(0.93,1.08,1./0.83,1.22,1.23)
> or.pro

[1] 0.930000 1.080000 1.204819 1.220000 1.230000
```

The corresponding  $P$  – values are

```
> p <- c(0.6,0.84,0.40,0.25,0.07)
```

Let us find log-ORs

```
> logor.pro <- log(or.pro)
> logor.pro

[1] -0.07257069 0.07696104 0.18632958 0.19885086 0.20701417
```

Corresponding squared standard errors are

```
> s2 <- logor.pro*logor.pro/qchisq(1-p,1)
> s2

[1] 0.01915121 0.14531060 0.04901514 0.02988102 0.01305349
```

and weights are

```
> w <- 1/s2
> w

[1] 52.216009 6.881811 20.401860 33.466060 76.607876
```

Thus the pooled estimate of log-OR is

```
> p.logor.pro <- sum(w*logor.pro)/sum(w)
> p.logor.pro
```

```
[1] 0.1216172
```

and the standard error is

```
> p.s <- 1/sqrt(sum(w))
> p.s
```

```
[1] 0.07262917
```

Thus the pooled estimate of Odds Ratio from association between type 2 diabetes and "Ala" allele is

```
> exp(p.logor.pro)
```

```
[1] 1.129322
```

and the 95% confidence interval is

```
> exp(p.logor.pro-1.96*p.s)
```

```
[1] 0.9794776
```

```
> exp(p.logor.pro+1.96*p.s)
```

```
[1] 1.30209
```

The  $\chi^2$  test for association and corresponding  $P$  – value are

```
> p.chi2 <- (p.logor.pro/p.s)^2
> p.chi2
```

```
[1] 2.803937
```

```
> p.pval <- 1-pchisq(p.chi2,1)
> p.pval
```

```
[1] 0.09403318
```

Z-score pooling though may be more appropriate method for such differentially designed studies (e.g. control groups are very different). To get Z-score pooling working, we need first find Z-scores from P-values

```
> p <- c(0.6,0.84,0.40,0.25,0.07)
> z <- sqrt(qchisq(1-p,1))
> z
```

```
[1] 0.5244005 0.2018935 0.8416212 1.1503494 1.8119107
```

and assign the right sign (let "+" is for the risk effect of "Pro").

```
> effsig <- c(-1,1,1,1,1)
> z <- z*effsig
> z
```

```
[1] -0.5244005 0.2018935 0.8416212 1.1503494 1.8119107
```

Now, we need to assign weights to the studies as

```
> n <- c(306, 71, 164, 242, 471)
> w <- sqrt(n)
```

and the pooled estimate of  $Z$  and corresponding  $P$  – value are

```
> zpoo <- sum(w*z)/sqrt(sum(w^2))
> zpoo
```

```
[1] 1.709151
```

```
> 1-pchisq(zpoo*zpoo, 1)
```

```
[1] 0.08742297
```

As you can see the results are almost identical to the previous obtained with inverse variance pooling.

## Chapter 11

# Analysis of selected region

Small data set 'srdta', which is part of the `GenABEL-package` library, will be used in this section. Start R and load the `GenABEL-package` and the data with

```
> library(GenABEL)
> data(srdta)
```

### 11.1 Exploring linkage disequilibrium

See help for `r2fast`.

### 11.2 Haplotype analysis

Use

```
> gtforld <- as.hsgeno(srdta[,1:5])
```

to convert part of your SNPs to `haplo.stats` format.

You can also use interface function to do sliding widow analysis

```
> h2 <- scan.haplo("qt1~CRSNP",srdta,snps=c(1:5))
```

### 11.3 Analysis of interactions

See help for `scan.haplo.2D` and `scan.glm.2D`



## Appendix A

# Importing data to GenABEL-package

As described in section 4.1, **GenABEL-package** `gwaa.data`-class consist of phenotypic data frame and an object of `snp.data`-class, which contains all genetic data. To import data to **GenABEL-package**, you need to prepare two files: one containing the phenotypic, and the other containing genotypic data.

The phenotype file relates study subject IDs with values of covariates and outcomes. In the phenotypic data file, the first line gives a description (variable name) of the data contained in a particular column; the names should better be unique, otherwise R will change them.

The first column of the phenotype file **must** contain the subjects' unique ID, named "id". The IDs listed here, and in the genotypic data file, must be the same. It is recommended that the id names are given in quotation marks (see example below), which will save you a possible troubles with e.g. leading zeros.

There also should also be a column named "sex" and giving sex information (0 = female, 1 = male). Other columns in the file should contain phenotypic information.

Missing values should be coded with "NA"; binary traits should have values 0 or 1.

All subjects present in the genotypic files **must** be listed in the phenotypic file as well, because sex information provided by the phenotypic file is an essential part of the genotypic QC procedure.

An example of few first lines of a phenotype file is as follows:

id	sex	age	bt1	qt	qt1
"cd289982"	0	30.33	NA	NA	3.93
"cd325285"	0	36.514	1	0.49	3.61
"cd357273"	1	37.811	0	1.65	5.30
"cd872422"	1	20.393	0	1.95	4.07
"cd1005389"	1	28.21	1	0.35	3.90

This file tells us that, for example, person 325286 is female (0 in second column), and she has "1" (usually this means a "case") value for the trait "bt1", so on. Person 289982 has measurements only for sex, age and qt1, while other measurements are missing (NA, Not Available).

If you need to add phenotypes to a `gwaa.data-class` object already created, you can use function `add.phdata`. This function allows you to add variables contained in some data frame to the existing `data@phdata` object. The data frame to be added should contain "id" variable, identical to that existing in the object, and **should not** contain any other variables with names identical to these already existing.

The second file you need should contain genotypic data. As described in section 4.1 ("General description of `gwaa.data-class`", page 77), `GenABEL-package` `snp.data-class` contains different types of information. For every SNP, information on map position, chromosome, and strand should be provided. For every person, every SNP genotype should be provided. `GenABEL-package` provides a number of functions to convert these data from different formats to the internal `GenABEL-package` raw format. We will first consider our preferred format, which we informally call "Illumina"-like.

## A.1 Converting from preferred format

We will consider use of `convert.snp.illumina` procedure; details of other procedures are given later. Note that what we call "illumina" format is not really a proprietary format from that company, it is just one of the possible text output formats from the Illumina BeadStudio; similar formats are accepted/generated by HapMap and Affymetrix.

The file of the "Illumina" format contains SNPs in rows and IDs in columns. The first line is a "header", containing column names. The first three columns should contain information on SNP name, chromosome, and position. There is an optional (though highly recommended!) fourth column, containing strand information (acceptable codes: "+", "-", "u", the last stands for "unknown"). After that column, each of the residual ones corresponds to an individual, with ID as the column name. Genotypes should be presented by two consecutive characters (no separator).

An example of few first lines of the "illumina" genotypic file is as follows:

name	chr	pos	strand	"cd289982"	"cd325285"	"cd357273"	"cd872422"	"cd1005389"
rs1001	1	1235	+	AA	AG	AG	AA	GG
rs6679	9	2344	+	GT	GG	GG	TG	GG
rs2401	22	3455	+	AA	CC	CC	CC	AC
rs123	X	32535	-	TT	GT	TT	TT	TT
rs6679	XY	2344	-	GT	GG	GG	TG	GG
rs876	Y	23556	+	OO	OO	TT	GG	TT
mitoA1	mt	24245	-	AA	CC	OO	OO	OO

It is clear that is not quite conventional Illumina file – because in BeadStudio the alleles are reported using the "top" strand; rather, this is an Affymetrix or HapMap-type of a file. Anyways, this file contains all required genotypic information, and this file format is the preferred one for import. Assume that the file with the genotypic data is called "gen0.illu", and is stored in the directory "RData".

First, start R and load `GenABEL-package`:

```
> library(GenABEL)
```



You can convert the data to **GenABEL**-package raw format by

```
> convert.snp.illumina(inf="RData/gen0.illu",out="RData/gen0i.raw",strand="file")
```

```
Reading genotypes from file 'RData/gen0.illu' ...
Writing to file 'RData/gen0i.raw' ...
... done.
```

Here is the content of the converted file "gen0i.raw" – internal raw data representation:

```
#GenABEL raw data version 0.1
"cd289982" "cd325285" "cd357273" "cd872422" "cd1005389"
rs1001 rs6679 rs2401 rs123 rs6679 rs876 mitoA1
1 9 22 X XY Y mt
1235 2344 3455 32535 2344 23556 24245
04 0c 0f 08 0c 08 0f
01 01 01 02 02 01 02
69 c0
96 40
d5 80
65 40
96 40
07 40
d0 00
```

Note the option **strand="file"** – it is telling **GenABEL**-package that strand information is provided in the file.

At this moment, you can load the data into **GenABEL**-package. Assume that the phenotypic file described above is called "phe0.dat" and the converted genotypic file in the raw **GenABEL**-package format is called "gen0i.raw". You can load the data using the command

```
> df <- load.gwaa.data(phe="RData/phe0.dat",gen="RData/gen0i.raw",force=T)
```

```
ids loaded...
marker names loaded...
chromosome data loaded...
map data loaded...
allele coding data loaded...
strand data loaded...
genotype data loaded...
snp.data object created...
assignment of gwaa.data object FORCED; X-errors were not checked!
```

The option "force=TRUE" tells that **GenABEL**-package should load the data even if it finds sex errors.

You can inspect the loaded data; let us first look into phenotypic data by

```
> df@phdata
```

	id	sex	age	bt1	qt	qt1
cd289982	cd289982	0	30.330	NA	NA	3.93
cd325285	cd325285	0	36.514	1	0.49	3.61
cd357273	cd357273	1	37.811	0	1.65	5.30
cd872422	cd872422	1	20.393	0	1.95	4.07
cd1005389	cd1005389	1	28.210	1	0.35	3.90

... and then check that the genotypes have imported right:

```
> g0 <- as.character(df@gtdata)
> g0
```

	rs1001	rs6679	rs2401	rs123	rs6679	rs876	mitoA1
cd289982	"A/A"	"G/T"	"A/A"	"T/T"	"G/T"	NA	"A/A"
cd325285	"A/G"	"G/G"	"C/C"	"T/G"	"G/G"	NA	"C/C"
cd357273	"A/G"	"G/G"	"C/C"	"T/T"	"G/G"	"T/T"	NA
cd872422	"A/A"	"G/T"	"C/C"	"T/T"	"G/T"	"G/G"	NA
cd1005389	"G/G"	"G/G"	"C/A"	"T/T"	"G/G"	"T/T"	NA

```
> as.character(df@gtdata@strand)
```

	rs1001	rs6679	rs2401	rs123	rs6679	rs876	mitoA1
	"+"	"+"	"+"	"-"	"-"	"+"	"-"

```
> as.character(df@gtdata@coding)
```

	rs1001	rs6679	rs2401	rs123	rs6679	rs876	mitoA1
	"AG"	"GT"	"CA"	"TG"	"GT"	"TG"	"CA"

In a real Illumina file, a coding on the TOP strand is supplied. Then, the file will normally look like

name	chr	pos	"cd289982"	"cd325285"	"cd357273"	"cd872422"	"cd1005389"
rs1001	1	1235	AA	AG	AG	AA	GG
rs6679	9	2344	GT	GG	GG	TG	GG
rs2401	22	3455	AA	CC	CC	CC	AC
rs123	X	32535	TT	GT	TT	TT	TT
rs6679	XY	2344	GT	GG	GG	TG	GG
rs876	Y	23556	00	00	TT	GG	TT
mitoA1	mt	24245	AA	CC	00	00	00

and the conversion command will be

```
> convert.snp.illumina(inf="RData/gen0.illuwos",out="RData/gen0iwos.raw",strand="+")
```

```
Reading genotypes from file 'RData/gen0.illuwos' ...
Writing to file 'RData/gen0iwos.raw' ...
... done.
```

In this particular data set, after conversion, the "+" strand will actually mean not "forward", but TOP – something you should remember for this particular data. The resulting file will look like this:

You can load the data with

```
> df <- load.gwaa.data(phe="RData/phe0.dat",gen="RData/genOiwos.raw",force=T)

ids loaded...
marker names loaded...
chromosome data loaded...
map data loaded...
allele coding data loaded...
strand data loaded...
genotype data loaded...
snp.data object created...
assignment of gwaa.data object FORCED; X-errors were not checked!
```

Obviously, the "strand" is always "+" (here it means TOP):

```
> g1 <- as.character(df@gtdata)
> g1

      rs1001 rs6679 rs2401 rs123 rs6679 rs876 mitoA1
cd289982 "A/A"  "G/T"  "A/A"  "T/T" "G/T"  NA   "A/A"
cd325285 "A/G"  "G/G"  "C/C"  "T/G" "G/G"  NA   "C/C"
cd357273 "A/G"  "G/G"  "C/C"  "T/T" "G/G"  NA   "T/T"
cd872422 "A/A"  "G/T"  "C/C"  "T/T" "G/T"  NA   "G/G"
cd1005389 "G/G"  "G/G"  "C/A"  "T/T" "G/G"  NA   "T/T"

> as.character(df@gtdata@strand)

rs1001 rs6679 rs2401 rs123 rs6679 rs876 mitoA1
      "+"      "+"      "+"      "+"      "+"      "+"      "+"

> as.character(df@gtdata@coding)

rs1001 rs6679 rs2401 rs123 rs6679 rs876 mitoA1
      "AG"    "GT"    "CA"    "TG"    "GT"    "TG"    "CA"
```

We can see that the genotypes are identical to ones we imported previously, as should be the case:

```
> g0 == g1

      rs1001 rs6679 rs2401 rs123 rs6679 rs876 mitoA1
cd289982   TRUE   TRUE   TRUE   TRUE   TRUE   NA   TRUE
cd325285   TRUE   TRUE   TRUE   TRUE   TRUE   NA   TRUE
cd357273   TRUE   TRUE   TRUE   TRUE   TRUE   TRUE  NA
cd872422   TRUE   TRUE   TRUE   TRUE   TRUE   TRUE  NA
cd1005389   TRUE   TRUE   TRUE   TRUE   TRUE   TRUE  NA
```

## A.2 Converting PLINK tped files

PLINK TPED (transposed-pedigree) format bears notable similarity to what we call "Illumina" format, with few exceptions: (1) there is no header line giving field names (and therefore IDs are stored in a separate file) (2) the first column gives chromosome, second – SNP name, third *genetic* map (usually kept as

zeroes), the fourth – physical position, and, starting with the fifth column, genotypic data are listed, (3) finally, within a genotypes, alleles are separated with a space. In TPED format, the data we already worked with would look like

1	rs1001	0	1235	A A	A G	A G	A A	G G
9	rs6679	0	2344	G T	G G	G G	T G	G G
22	rs2401	0	3455	A A	C C	C C	C C	A C
X	rs123	0	32535	T T	G T	T T	T T	T T
XY	rs6679	0	2344	G T	G G	G G	T G	G G
Y	rs876	0	23556	0 0	0 0	T T	G G	T T
mt	mitoA1	0	24245	A A	C C	0 0	0 0	0 0

Obviously, a separate file is needed to keep correspondence between genotypes and IDs. This file emulated standard pedigree file without a header line. The file, conventionally called a TFAM-file, should contain six columns, corresponding to pedigree ID, ID, father, mother, sex, and affection. Only the second column is used by **GenABEL-package** – please make sure you use unique IDs. Consequently, it does not matter what pedigree ID, father/mother, sex, or affection status you assign in the file – the real information is coming from the phenotypic data file. The TFAM file for our data will look like this:

```
1 cd289982 0 0 1 0
1 cd325285 0 0 1 0
1 cd357273 0 0 1 0
1 cd872422 0 0 1 0
1 cd1005389 0 0 1 0
```

You can convert the data from PLINK TPED format to the **GenABEL-package** format using command `convert.snp.tped`:

```
> convert.snp.tped(tped="RData/gen0.tped",tfam="RData/gen0.tfam",out="RData/gen0tped.raw")

Reading individual ids from file 'RData/gen0.tfam' ...
... done. Read 5 individual ids from file 'RData/gen0.tfam'
Reading genotypes from file 'RData/gen0.tped' ...
...done. Read 7 SNPs from file 'RData/gen0.tped'
Writing to file 'RData/gen0tped.raw' ...
... done.
```

and load the data with

```
> df <- load.gwaa.data(phe="RData/phe0.dat",gen="RData/gen0tped.raw",force=T)

ids loaded...
marker names loaded...
chromosome data loaded...
map data loaded...
allele coding data loaded...
strand data loaded...
genotype data loaded...
snp.data object created...
assignment of gwaa.data object FORCED; X-errors were not checked!
```

Obviously, the "strand" is always "+" (meaning TOP):

```
> g1 <- as.character(df@gtdata)
> g1
```

	rs1001	rs6679	rs2401	rs123	rs6679	rs876	mitoA1
cd289982	"A/A"	"G/T"	"A/A"	"T/T"	"G/T"	NA	"A/A"
cd325285	"A/G"	"G/G"	"C/C"	"T/G"	"G/G"	NA	"C/C"
cd357273	"A/G"	"G/G"	"C/C"	"T/T"	"G/G"	"T/T"	NA
cd872422	"A/A"	"G/T"	"C/C"	"T/T"	"G/T"	"G/G"	NA
cd1005389	"G/G"	"G/G"	"C/A"	"T/T"	"G/G"	"T/T"	NA

```
> as.character(df@gtdata@strand)
```

rs1001	rs6679	rs2401	rs123	rs6679	rs876	mitoA1
"+"	"+"	"+"	"+"	"+"	"+"	"+"

```
> as.character(df@gtdata@coding)
```

rs1001	rs6679	rs2401	rs123	rs6679	rs876	mitoA1
"AG"	"GT"	"CA"	"TG"	"GT"	"TG"	"CA"

We can see that the genotypes are identical to ones we imported previously, as should be the case:

```
> g0 == g1
```

	rs1001	rs6679	rs2401	rs123	rs6679	rs876	mitoA1
cd289982	TRUE	TRUE	TRUE	TRUE	TRUE	NA	TRUE
cd325285	TRUE	TRUE	TRUE	TRUE	TRUE	NA	TRUE
cd357273	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	NA
cd872422	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	NA
cd1005389	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	NA

### A.3 Converting linkage-like files

Linkage-like files, also known as pre-makeped files, or pedigree files, represent a historic format which dates back to the time when only few markers could be typed – thus the number of subjects was usually greater than the number of markers. In that situation, it was natural and obvious to keep IDs in rows and markers in columns. In the first six columns, standard linkage-like file would contain pedigree ID, ID, father's ID, mother's ID, sex (coded as 1 = male and 2 = female), and affection status (0 = unknown, 1 = unaffected, 2 = affected). In the following columns, genotypic information is provided. Alleles of the same genotype could be separated by a space, or by a slash ("/"). Thus the data we are working with could be presented as

1	cd289982	0	0	1	0	A A	G T	A A	T T	G T	0 0	A A
1	cd325285	0	0	1	0	A G	G G	C C	G T	G G	0 0	C C
1	cd357273	0	0	1	0	A G	G G	C C	T T	G G	T T	0 0
1	cd872422	0	0	1	0	A A	T G	C C	T T	T G	G G	0 0
1	cd1005389	0	0	1	0	G G	G G	A C	T T	G G	T T	0 0

As you can see, this file misses header line, and information what are the SNP names, position, etc. should be provided in a separate MAP-file. **GenABEL**-package accepts map in Merlin format, and an extended format. A map in Merlin format consist of header line, giving column names, and three columns with chromosome, name and position information, for example:

```
chr  name    pos
1    rs1001   1235
9    rs6679   2344
22   rs2401   3455
X    rs123    32535
XY   rs6679   2344
Y    rs876    23556
mt   mitoA1   24245
```

The data can be converted to the internal **GenABEL**-package format with

```
> convert.snp.ped(pedfile="RData/gen0.ped",mapfile="RData/map0.dat",out="RData/gen0pedwos.raw")

Reading map from file 'RData/map0.dat' ...
... done. Read positions of 7 markers from file 'RData/map0.dat'
Reading genotypes from file 'RData/gen0.ped' ...
...done. Read information for 5 people from file 'RData/gen0.ped'
Analysing marker information ...
Writing to file 'RData/gen0pedwos.raw' ...
... done.
```

and loaded with

```
> df <- load.gwaa.data(phe="RData/phe0.dat",gen="RData/gen0pedwos.raw",force=T)

ids loaded...
marker names loaded...
chromosome data loaded...
map data loaded...
allele coding data loaded...
strand data loaded...
genotype data loaded...
snp.data object created...
assignment of gwaa.data object FORCED; X-errors were not checked!
```

We can inspect the genotypic data and check that conversion results are identical to previous runs with

```
> g1 <- as.character(df@gtdata)
> g1
```

	rs1001	rs6679	rs2401	rs123	rs6679	rs876	mitoA1
cd289982	"A/A"	"G/T"	"A/A"	"T/T"	"G/T"	NA	"A/A"
cd325285	"A/G"	"G/G"	"C/C"	"T/G"	"G/G"	NA	"C/C"
cd357273	"A/G"	"G/G"	"C/C"	"T/T"	"G/G"	"T/T"	NA
cd872422	"A/A"	"G/T"	"C/C"	"T/T"	"G/T"	"G/G"	NA
cd1005389	"G/G"	"G/G"	"C/A"	"T/T"	"G/G"	"T/T"	NA

```
> as.character(df@gtdata@strand)

rs1001 rs6679 rs2401 rs123 rs6679 rs876 mitoA1
      "+"      "+"      "+"      "+"      "+"      "+"      "+"

> as.character(df@gtdata@coding)

rs1001 rs6679 rs2401 rs123 rs6679 rs876 mitoA1
      "AG"     "GT"     "CA"     "TG"     "GT"     "TG"     "CA"

> g0 == g1

           rs1001 rs6679 rs2401 rs123 rs6679 rs876 mitoA1
cd289982   TRUE   TRUE   TRUE   TRUE   TRUE   NA   TRUE
cd325285   TRUE   TRUE   TRUE   TRUE   TRUE   NA   TRUE
cd357273   TRUE   TRUE   TRUE   TRUE   TRUE   TRUE  NA
cd872422   TRUE   TRUE   TRUE   TRUE   TRUE   TRUE  NA
cd1005389  TRUE   TRUE   TRUE   TRUE   TRUE   TRUE  NA
```

If you are willing to import strand information, you can make use of the *extended* map format. In this format the strand information is added to the map-file:

chr	name	pos	strand	coding
1	rs1001	1235	+	AG
9	rs6679	2344	+	TG
22	rs2401	3455	+	AC
X	rs123	32535	-	GT
XY	rs6679	2344	-	GT
Y	rs876	23556	+	GT
mt	mitoA1	24245	-	AC

The data can be converted to the internal GenABEL-package format with

```
> convert.snp.ped(pedfile="RData/gen0.ped",mapfile="RData/emap0.dat",out="RData/gen0ped.raw",strand=file)

Reading map from file 'RData/emap0.dat' ...
... done. Read positions of 7 markers from file 'RData/emap0.dat'
Reading genotypes from file 'RData/gen0.ped' ...
...done. Read information for 5 people from file 'RData/gen0.ped'
Analysing marker information ...
Writing to file 'RData/gen0ped.raw' ...
... done.
```

Note that option `strand==file` was used to specify that the extended map format should be used. The data can be loaded with

```
> df <- load.gwaa.data(phe="RData/phe0.dat",gen="RData/gen0ped.raw",force=T)

ids loaded...
marker names loaded...
chromosome data loaded...
map data loaded...
```

```
allele coding data loaded...
strand data loaded...
genotype data loaded...
snp.data object created...
assignment of gwaa.data object FORCED; X-errors were not checked!
```

We can inspect the genotypic data and check that conversion results are identical to previous runs with

```
> g1 <- as.character(df@gtdata)
> g1
```

	rs1001	rs6679	rs2401	rs123	rs6679	rs876	mitoA1
cd289982	"A/A"	"G/T"	"A/A"	"T/T"	"G/T"	NA	"A/A"
cd325285	"A/G"	"G/G"	"C/C"	"T/G"	"G/G"	NA	"C/C"
cd357273	"A/G"	"G/G"	"C/C"	"T/T"	"G/G"	"T/T"	NA
cd872422	"A/A"	"G/T"	"C/C"	"T/T"	"G/T"	"G/G"	NA
cd1005389	"G/G"	"G/G"	"C/A"	"T/T"	"G/G"	"T/T"	NA

```
> as.character(df@gtdata@strand)
```

rs1001	rs6679	rs2401	rs123	rs6679	rs876	mitoA1
"+"	"+"	"+"	"-"	"-"	"+"	"-"

```
> as.character(df@gtdata@coding)
```

rs1001	rs6679	rs2401	rs123	rs6679	rs876	mitoA1
"AG"	"GT"	"CA"	"TG"	"GT"	"TG"	"CA"

```
> g0 == g1
```

	rs1001	rs6679	rs2401	rs123	rs6679	rs876	mitoA1
cd289982	TRUE	TRUE	TRUE	TRUE	TRUE	NA	TRUE
cd325285	TRUE	TRUE	TRUE	TRUE	TRUE	NA	TRUE
cd357273	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	NA
cd872422	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	NA
cd1005389	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	NA

## A.4 Converting from MACH format

The data from MACH format can be converted by using `convert.snp.mach`. This function actually calls `convert.snp.ped` in specific format. MACH software is widely used for SNP imputations. For our needs we consider two files produced by MACH: pedigree file with (the imputed) genotypic data, and info-file, containing information about quality of imputations for particular SNP.

SEE HELP FOR `convert.snp.mach` for further details.

## A.5 Converting from text format



## Appendix B

# GenABEL internals

### B.1 Internal structure of `gwaa.data-class`

Start R and load **GenABEL**-package library using command

```
> library(GenABEL)
```

After that, load the data with the command

```
> data(srdta)
```

The object you have loaded, `srdta`, belongs to the `gwaa.data` class. This is a special class developed to facilitate GWA analysis.

In GWA analysis, different types of data are used. These include the phenotypic and genotypic data on the study participants and chromosome and location of every SNP. For every SNP, it is desirable to know the details of coding (what are alleles? – A, T, G, C? – and what is the strand – '+' or '-', 'top' or 'bot'? – this coding is for).

One could attempt to store all phenotypes and genotypes together in a single table, using, e.g. one row per study subject; than the columns will correspond to study phenotypes and SNPs. For a typical GWA data set, this would lead to a table of few thousands rows and few hundreds of thousands of columns. Such a format is generated when one downloads HapMap data for a region. To store GWA data in such tables internally, within R, proves to be inefficient. In **GenABEL**-package, special data class, `gwaa.data-class` is used to store GWA data. The structure of this data class is shown at the figure B.1.

An object of some class has "slots" which may contain actual data or objects of other classes. The information stored at a particular `slot` of an `object` can be accessed by command `object@slot`.

At the first level, a `gwaa.data-class` object has slot `phdata`, which contains all phenotypic information in a data frame (`data.frame-class` object). The rows of this data frame correspond to study subjects, and the columns correspond to the variables. There are two default variables, which are always present in `phdata`. The first of these is "id", which contains study subject identification code. This identification code can be arbitrary character, but every person must be coded with an unique ID. The second default variable is "sex", where males are coded with ones ("1") and females are coded with zero ("0"). It is important

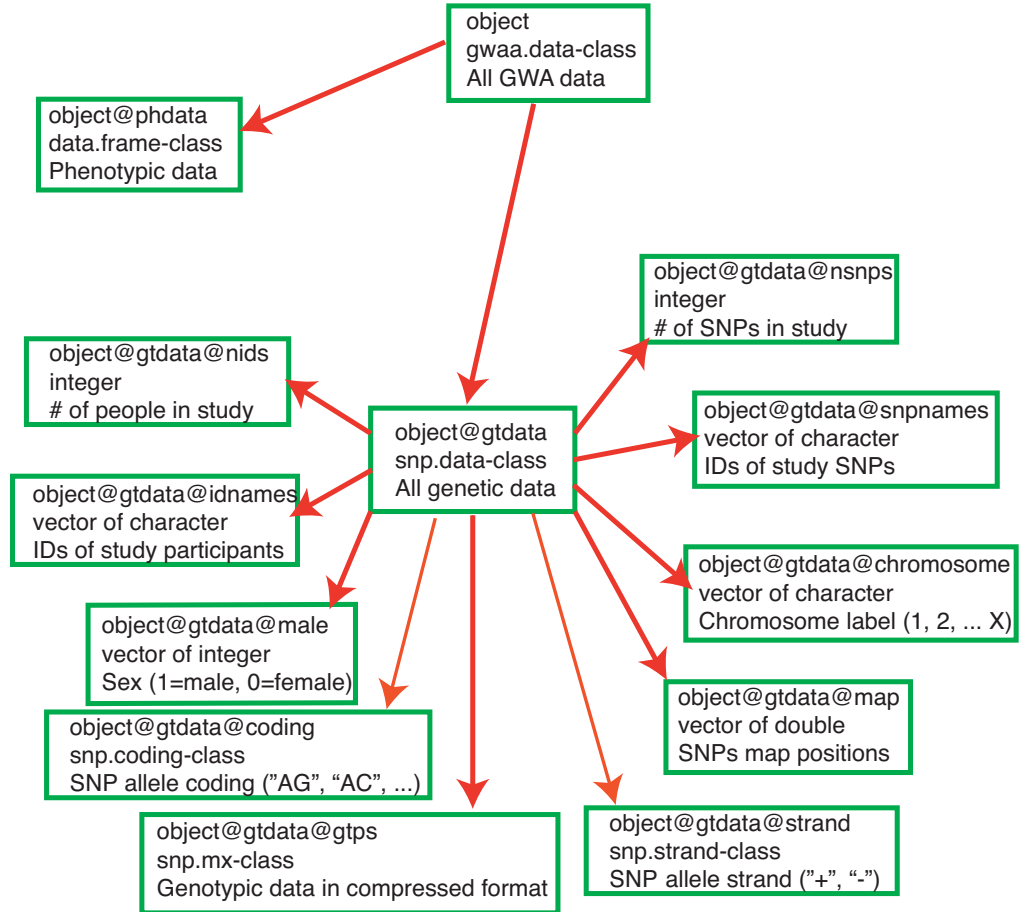


Figure B.1: Structure of gwaa.data-class. In every box, first line contains the object and slot names, second line describes the class of this object, and third line describes what information is contained.

to understand that this data frame is not supposed to be directly modified by the user. In particular, it is extremely important to remember that one should not directly add subjects to the table, change the values of "id" and "sex", and change the order of subjects in `phdata` unless this one is really understands the way **GenABEL-package** works. One also should not run such data manipulation functions as `merge`, `cbind` and `rbind` – exactly because they may change the number of subjects or interfere with the order. On the other hand, it is OK to add more variables to the data frame through direct computations, for example, if one wishes to add computed body mass index, it is OK to run the command like

```
obj@phdata$bmi <- obj@phdata$weight/((obj@phdata$height)2)
```

To add many variables to `phdata`, special GenABEL function `add.phdata` should be used.

The other slot of an object of `gwaa.data-class` is slot `gtdata`, which contains all GWA genetic information in an object of class `snp.data` class (figure B.1). This class, in turn, has slots `nids`, containing the number of study subjects, `idnames`, containing all ID names of these subjects, `nsnps`, containing the number of SNPs typed, `snpnames`, containing the SNP names, `chromosome`, containing the name of the chromosome the SNPs belong to and slot `map` with map position of SNPs, and slot `male`, containing the sex code for the subjects (1=male, 0=female). The latter is identical to the "sex" variable contained in the `phdata`, but is duplicated here because many operations with purely genetic data, in particular these concerning analysis of sex chromosomes, depend on the sex. The strand information is presented in the slot `strand`. **GenABEL-package** codes strand as "+" (forward), "-" (reverse) or "u" (unknown). Of cause, if you prefer top/bottom coding, this information may be stored in the same form – you will just need to remember that "+" corresponds to e.g. "top", and "-" to "bottom" strand. The allelic coding is presented in slot `coding`. Coding for every allele is presented with a pair of characters, for example "AG". Thus, for such polymorphism, you may expect "AA", "AG" and "GG" genotypes to be found in population. The order (that is "AG" vs "GA") is important – the first allele reported is the one which will be used as a reference in association analysis, and thus the effects are reported for the second allele. To avoid memory overheads, the strand and coding information is internally stored as `snp.strand-class` and `snp.coding-class`. Information can be converted to human-readable format using `as.character` function.

If, for example, you would like to know, how many SNPs were included in the study (slot `nsnps` of the slot `gtdata` of `srdata`), you need to run command

```
> srdata@gtdata@nsnps
```

```
[1] 833
```

Thus, 833 SNPs were typed in the study. You can access information stored in any slot in this manner.

You may want to read the general **GenABEL-package** man page using `help(GenABEL)`. To see help on `gwaa.data-class`, you can use `help("gwaa.data-class")` (mind the quotation marks!).

**Summary:**

- An object of some class has "slots" which may contain actual data or objects of other classes. The information stored at a particular `slot` of an object can be accessed by command `object@slot`.
- `GenABEL-package` uses special data class, `gwaa.data-class`, to store GWA data.

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