

# Preparing case-parent trio data and detecting disease-associated SNP interactions with `trio`

Qing Li, Holger Schwender, and Ingo Ruczinski

## 1 Introduction

The R package `trio` contains functions for performing genotypic transmission disequilibrium tests (gTDTs) for testing whether the distributions of individual SNPs (Schaid, 1996), two-way interactions of SNPs (Cordell, 2002; Cordell *et al.*, 2004), or interactions between SNPs and binary environmental variables differ between the cases, i.e. the children affected by a disease, and the pseudo-controls derived from the parents' genotypes.

Furthermore, `trio` provides functionalities relevant for the analysis of case-parent trio data with *trio logic regression* (Li *et al.*, 2010). Two major features are implemented in this package: functions that aid in the transformation of the trio data from standard linkage files (ped format) or genotype format into objects suitable as input for trio logic regression, and a framework that allows for the simulation of case-parent data, where the risk of disease is specified by (higher order) SNP interactions.

In Section 2 of this vignette, it is shown how family-based data stored in a linkage/ped file can be read into R and transformed into a format suitable for the application of the functions for performing the genotypic TDTs, whereas Section 3 contains examples for the application of these gTDT functions to individual SNPs, two-way SNP interactions, and gene-environment interactions.

Section 4 is devoted to the steps relevant for data processing, to derive a matrix suitable as input for trio logic regression, starting from a linkage or genotype file which possibly contains missing data and/or Mendelian errors. We give some examples how missing data can be addressed using haplotype-based imputation. The haplotype information can be specified by the user, or when this information is not readily available, automatically inferred. The haplotype blocks are also relevant in the delineation of the genotypes for the pseudo-controls, as the linkage disequilibrium (LD) structure observed in the parents is taken into account in this process. While this function is intended to generate complete case-pseudo-control data as input for trio logic regression, an option to simply return the completed trio data is also available.

For the estimation of the haplotype structure that might be used in the functions described in Section 4, the R package `trio` also contains functions for computing and plotting the pairwise LD values and for detecting LD blocks. In Section 5, it is described how the pairwise values of the LD measures  $D'$  and  $r^2$  can be computed with the function `getLD()`, and how the  $D'$  values can be employed to estimate haplotype blocks with the algorithm of Gabriel *et al.* (2002).

Finally, Section 6 of the vignette explains in more detail how to set up simulations of case-parent trio data, where the risk of disease is specified by SNP interactions. The most time-consuming step for these types of simulations is the generation of mating tables and the respective probabilities. The mating table information, however, can be stored, which allows for fast simulations when replicates of the case-parent trio data are generated.

## 2 Preparing data for the genotypic TDTs

Case-parent trio data are typically stored in a ped file. The first six columns in such a ped file, which is also referred to as linkage file, identify the family structure of the data, and the phenotype. It is assumed that only one phenotype variable (column 6) is used. The

object `trio.ped1`, available in the R package, is an example of a data set in `ped` format. It contains information for 10 SNPs in 100 trios. Besides the variables providing information on the family structure and the phenotypes (columns 1–6), each SNP is encoded in two variables denoting the alleles.

```
> library(trio)
> data(trio.data)
> str(trio.ped1)
```

```
'data.frame':      300 obs. of  26 variables:
 $ famid   : int  10001 10001 10001 10002 10002 10002 10003 10003 10003 10004 ...
 $ pid     : int  1 2 3 1 2 3 1 2 3 1 ...
 $ fatid   : int  0 0 1 0 0 1 0 0 1 0 ...
 $ motid   : int  0 0 2 0 0 2 0 0 2 0 ...
 $ sex     : int  1 2 2 1 2 1 1 2 1 1 ...
 $ affected: int  0 0 2 0 0 2 0 0 2 0 ...
 $ snp1_1  : int  1 1 1 1 1 1 1 1 1 1 ...
 $ snp1_2  : int  1 1 1 1 1 1 1 1 1 2 ...
 $ snp2_1  : int  1 2 1 1 2 1 1 1 1 1 ...
 $ snp2_2  : int  1 2 2 1 2 2 1 1 1 1 ...
 $ snp3_1  : int  1 1 1 2 1 1 1 1 1 1 ...
 $ snp3_2  : int  2 1 2 2 1 2 1 2 1 2 ...
 $ snp4_1  : int  1 1 1 2 1 1 1 1 1 1 ...
 $ snp4_2  : int  2 1 2 2 1 2 1 2 1 2 ...
 $ snp5_1  : int  1 2 1 1 2 1 1 1 1 1 ...
 $ snp5_2  : int  2 2 2 1 2 2 2 1 1 1 ...
 $ snp6_1  : int  1 1 1 2 1 1 1 1 1 1 ...
 $ snp6_2  : int  2 1 2 2 1 2 1 2 1 2 ...
 $ snp7_1  : int  1 1 1 1 1 1 1 1 1 1 ...
 $ snp7_2  : int  1 1 1 1 1 1 1 1 1 1 ...
 $ snp8_1  : int  1 1 1 1 1 1 1 1 1 1 ...
 $ snp8_2  : int  1 1 1 1 1 1 1 1 1 1 ...
 $ snp9_1  : int  1 1 1 1 1 1 1 1 1 1 ...
 $ snp9_2  : int  1 1 1 1 1 1 1 1 1 1 ...
 $ snp10_1 : int  1 1 1 1 1 1 1 1 1 1 ...
 $ snp10_2 : int  1 1 1 2 1 1 1 1 1 2 ...
```

```
> trio.ped1[1:10,1:12]
```

|   | famid | pid | fatid | motid | sex | affected | snp1_1 | snp1_2 | snp2_1 | snp2_2 | snp3_1 | snp3_2 |
|---|-------|-----|-------|-------|-----|----------|--------|--------|--------|--------|--------|--------|
| 1 | 10001 | 1   | 0     | 0     | 1   | 0        | 1      | 1      | 1      | 1      | 1      | 2      |
| 2 | 10001 | 2   | 0     | 0     | 2   | 0        | 1      | 1      | 2      | 2      | 1      | 1      |
| 3 | 10001 | 3   | 1     | 2     | 2   | 2        | 1      | 1      | 1      | 2      | 1      | 2      |
| 4 | 10002 | 1   | 0     | 0     | 1   | 0        | 1      | 1      | 1      | 1      | 2      | 2      |
| 5 | 10002 | 2   | 0     | 0     | 2   | 0        | 1      | 1      | 2      | 2      | 1      | 1      |
| 6 | 10002 | 3   | 1     | 2     | 1   | 2        | 1      | 1      | 1      | 2      | 1      | 2      |
| 7 | 10003 | 1   | 0     | 0     | 1   | 0        | 1      | 1      | 1      | 1      | 1      | 1      |
| 8 | 10003 | 2   | 0     | 0     | 2   | 0        | 1      | 1      | 1      | 1      | 1      | 2      |

|    |       |   |   |   |   |   |   |   |   |   |   |   |
|----|-------|---|---|---|---|---|---|---|---|---|---|---|
| 9  | 10003 | 3 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 |
| 10 | 10004 | 1 | 0 | 0 | 1 | 0 | 1 | 2 | 1 | 1 | 1 | 2 |

If not already available as data frame or matrix in the R workspace, trio data can be read into R using the function `read.pedfile()`. If we, for example, assume that the working directory of the current R session contains a file called "pedfile.ped" (this file is actually not available in `trio`, we just assume that such a file exists in the working directory), then this file can be read into R by calling

```
> ped <- read.pedfile("pedfile.ped")
```

If the arguments `coded` and `first.row` of `read.pedfile()` are not specified by the user, `read.pedfile()` automatically tries to figure out how the alleles in the ped file are coded, and whether the first row contains the SNP names (`first.row = FALSE`) or the data for the first subject (`first.row = TRUE`). In the former case, `read.pedfile()` adds the SNP names (with extensions `.1` and `.2` to differ between the two alleles) to the respective columns of the read-in data frame.

For the applications of the functions for performing gTDTs (see Section 3), the trio data must be in a matrix in genotype format. In such a matrix, each column represents a SNP, which is coded by the number of minor alleles, and each block of 3 consecutive rows contains the genotypes of the father, the mother, and their offspring (in this order) of one specific trio. Missing values are allowed in this matrix, and need to be coded by `NA`. This matrix can either be generated from a data frame in ped format by employing the function `ped2geno()`, or more conveniently, by setting `p2g = TRUE` in `read.pedfile()`. Thus, a matrix in genotype format might be obtained from the above ped file by calling

```
> geno <- read.pedfile("pedfile.ped", p2g=TRUE)
```

The output of these functions just contains the matrix in genotype format, whereas `trio.check()` described in Section 4 additionally contains information about Mendelian

errors. Instead of checking for Mendelian errors in `ped2geno()` or `read.pedfile()`, such errors are removed SNP-wise in the functions for performing genotypic TDTs.

If, for example, the data frame `trio.ped1` should be transformed into a matrix in genotype format, `ped2geno()` can be applied to it. However, `ped2geno()` requires unique personal IDs (second column of `trio.ped1`) such that we first have to combine the family ID and the personal ID (which would be automatically done by `read.pedfile()`), and change the IDs of the fathers and mothers in columns 3 and 4 likewise.

```
> data(trio.data)
> trio.ped1[,2] <- paste(trio.ped1[,1], trio.ped1[,2], sep="_")
> ids <- trio.ped1[,3] != 0
> trio.ped1[ids,3] <- paste(trio.ped1[ids,1], trio.ped1[ids,3], sep="_")
> trio.ped1[ids,4] <- paste(trio.ped1[ids,1], trio.ped1[ids,4], sep="_")
> trio.ped1[1:5, 1:4]
```

|   | famid | pid     | fatid   | motid   |
|---|-------|---------|---------|---------|
| 1 | 10001 | 10001_1 | 0       | 0       |
| 2 | 10001 | 10001_2 | 0       | 0       |
| 3 | 10001 | 10001_3 | 10001_1 | 10001_2 |
| 4 | 10002 | 10002_1 | 0       | 0       |
| 5 | 10002 | 10002_2 | 0       | 0       |

Afterwards, `ped2geno()` can be applied to `trio.ped1`

```
> geno <- ped2geno(trio.ped1)
> geno[1:5,]
```

|         | SNP1 | SNP2 | SNP3 | SNP4 | SNP5 | SNP6 | SNP7 | SNP8 | SNP9 | SNP10 |
|---------|------|------|------|------|------|------|------|------|------|-------|
| 10001_1 | 0    | 0    | 1    | 1    | 1    | 1    | 0    | 0    | 0    | 0     |
| 10001_2 | 0    | 2    | 0    | 0    | 2    | 0    | 0    | 0    | 0    | 0     |
| 10001_3 | 0    | 1    | 1    | 1    | 1    | 1    | 0    | 0    | 0    | 0     |
| 10002_1 | 0    | 0    | 2    | 2    | 0    | 2    | 0    | 0    | 0    | 1     |
| 10002_2 | 0    | 2    | 0    | 0    | 2    | 0    | 0    | 0    | 0    | 0     |

The matrix `trio.gen1` is the genotype matrix corresponding to `trio.ped1`. So the genotypes in the output of `ped2geno()` are identical to `trio.gen1` (except for that the first two columns of `trio.gen1` contain the family ID and the personal ID).

```
> data(trio.data)
> trio.gen1[1:5, 3:12]
```

|   | snp1 | snp2 | snp3 | snp4 | snp5 | snp6 | snp7 | snp8 | snp9 | snp10 |
|---|------|------|------|------|------|------|------|------|------|-------|
| 1 | 0    | 0    | 1    | 1    | 1    | 1    | 0    | 0    | 0    | 0     |
| 2 | 0    | 2    | 0    | 0    | 2    | 0    | 0    | 0    | 0    | 0     |
| 3 | 0    | 1    | 1    | 1    | 1    | 1    | 0    | 0    | 0    | 0     |
| 4 | 0    | 0    | 2    | 2    | 0    | 2    | 0    | 0    | 0    | 1     |
| 5 | 0    | 2    | 0    | 0    | 2    | 0    | 0    | 0    | 0    | 0     |

```
> table(trio.gen1[,3:12] == geno)
```

```
TRUE
3000
```

### 3 Testing SNPs, pairs of SNPs, and GxE interactions

A single SNP or two-way interaction can be tested with a gTDT by employing the functions `tdt()` and `tdt2way()`. If we, for example, would like to test the first SNP in the matrix `mat.test` available in the R package `trio`, then this could be done by calling

```
> data(trio.data)
> tdt(mat.test[,1])
```

Genotypic TDT Based on 3 Pseudo Controls

Model Type: Additive

| Coef     | OR     | Lower  | Upper | SE     | Statistic | p-Value |
|----------|--------|--------|-------|--------|-----------|---------|
| -0.04256 | 0.9583 | 0.6396 | 1.436 | 0.2063 | 0.04255   | 0.8366  |

In this case, a conditional logistic regression is fitted, and the output of `tdt()` contains the parameter estimate `Coef` for the SNP in this model, the odds ratio `OR`, the `Lower` and `Upper` bound of the 95% confidence interval of this odds ratio, the standard error `SE` of the parameter estimate, the Wald Statistic for testing whether this SNP has an effect, and the corresponding `p-Value`.

By default, an additive effect is tested. It is, however, also possible to consider a dominant effect

```
> tdt(mat.test[,1], model="dominant")
```

Genotypic TDT Based on 3 Pseudo Controls

Model Type: Dominant

| Coef    | OR     | Lower  | Upper | SE     | Statistic | p-Value |
|---------|--------|--------|-------|--------|-----------|---------|
| -0.1134 | 0.8928 | 0.5219 | 1.527 | 0.2739 | 0.1713    | 0.679   |

or a recessive effect

```
> tdt(mat.test[,1], model="recessive")
```

Genotypic TDT Based on 3 Pseudo Controls

Model Type: Recessive

| Coef    | OR    | Lower  | Upper | SE     | Statistic | p-Value |
|---------|-------|--------|-------|--------|-----------|---------|
| 0.06502 | 1.067 | 0.5279 | 2.157 | 0.3591 | 0.03278   | 0.8563  |

Similarly the interaction between SNP1 and SNP2 in `mat.test` can be tested by

```
> tdt2way(mat.test[,1], mat.test[,2])
```

Genotypic TDT for Epistatic Interactions (Using 15 Pseudo Controls)

Likelihood Ratio Test:  
 Loglikelihood (with Interactions): -272.644  
 Loglikelihood (without IAs): -275.29  
 Test Statistic: 5.293  
 P-Value: 0.26

In this case, the interaction is tested for epistatic interactions as described in Cordell (2002). Thus, two conditional logistic regression models are fitted to the cases and the respective 15 matched pseudo-controls (i.e. the 15 possible, but not transmitted Mendelian genotype realizations, given the parents' genotypes at the two loci), one consisting of two dummy variables for each of the two SNPs, and the other additionally containing the four possible interactions of these dummy variables. The two fitted models are then compared by a likelihood ratio test, and the  $p$ -values are computed by approximation to a  $\chi^2$ -distribution with four degrees of freedom.

This is the recommended way to test the two-way interaction. `tdt2way()`, however, also provides a simpler test, in which the values of the SNPs (either coding for an additive – which is the default – for a recessive, or for a dominant model) are simply multiplied for each case and its 15 matched pseudo-controls, and a conditional logistic regression is applied to this interaction.

```
> tdt2way(mat.test[,1], mat.test[,2], epistatic=FALSE)
```

Genotypic TDT for Two-Way Interaction (Using 15 Pseudo Controls)

Model Type: Additive

|  | Coef    | OR    | Lower  | Upper | SE     | Statistic | p-Value |
|--|---------|-------|--------|-------|--------|-----------|---------|
|  | 0.02424 | 1.025 | 0.7806 | 1.345 | 0.1387 | 0.03052   | 0.8613  |

All SNPs represented by the columns of a matrix in genotype format can be tested with a gTDT by employing the function `colTDT()`. Thus, all SNPs in `mat.test` can be tested by calling

```
> tdt.out <- colTDT(mat.test)
> tdt.out
```

Genotypic TDT Based on 3 Pseudo Controls

Model Type: Additive

Top 5 SNPs:

|      | Coef     | OR     | Lower  | Upper | SE     | Statistic | p-Value | Trios |
|------|----------|--------|--------|-------|--------|-----------|---------|-------|
| SNP6 | 0.44895  | 1.5667 | 0.9910 | 2.477 | 0.2337 | 3.6908    | 0.05471 | 63    |
| SNP3 | -0.22884 | 0.7955 | 0.5103 | 1.240 | 0.2265 | 1.0209    | 0.31232 | 66    |
| SNP2 | -0.19671 | 0.8214 | 0.5561 | 1.213 | 0.1990 | 0.9772    | 0.32288 | 73    |
| SNP4 | -0.13353 | 0.8750 | 0.5783 | 1.324 | 0.2113 | 0.3994    | 0.52740 | 71    |
| SNP5 | 0.09764  | 1.1026 | 0.7148 | 1.701 | 0.2211 | 0.1950    | 0.65881 | 64    |

By default, the five top SNPs, i.e. the five SNPs with the lowest *p*-values, are shown ordered by their significance. The top three SNPs can be shown by

```
> print(tdt.out, 3)
```

Genotypic TDT Based on 3 Pseudo Controls

Model Type: Additive

Top 3 SNPs:

|      | Coef    | OR     | Lower  | Upper | SE     | Statistic | p-Value | Trios |
|------|---------|--------|--------|-------|--------|-----------|---------|-------|
| SNP6 | 0.4490  | 1.5667 | 0.9910 | 2.477 | 0.2337 | 3.6908    | 0.05471 | 63    |
| SNP3 | -0.2288 | 0.7955 | 0.5103 | 1.240 | 0.2265 | 1.0209    | 0.31232 | 66    |
| SNP2 | -0.1967 | 0.8214 | 0.5561 | 1.213 | 0.1990 | 0.9772    | 0.32288 | 73    |

If the integer specified in `print()` is larger than or equal to the number of SNPs in the input matrix, the statistics for all SNPs are displayed in the order of their appearance in this matrix.

```
> print(tdt.out, 10)
```

Genotypic TDT Based on 3 Pseudo Controls

Model Type: Additive

|      | Coef     | OR     | Lower  | Upper | SE     | Statistic | p-Value | Trios |
|------|----------|--------|--------|-------|--------|-----------|---------|-------|
| SNP1 | -0.04256 | 0.9583 | 0.6396 | 1.436 | 0.2063 | 0.04255   | 0.83658 | 72    |
| SNP2 | -0.19671 | 0.8214 | 0.5561 | 1.213 | 0.1990 | 0.97724   | 0.32288 | 73    |
| SNP3 | -0.22884 | 0.7955 | 0.5103 | 1.240 | 0.2265 | 1.02085   | 0.31232 | 66    |
| SNP4 | -0.13353 | 0.8750 | 0.5783 | 1.324 | 0.2113 | 0.39941   | 0.52740 | 71    |
| SNP5 | 0.09764  | 1.1026 | 0.7148 | 1.701 | 0.2211 | 0.19497   | 0.65881 | 64    |
| SNP6 | 0.44895  | 1.5667 | 0.9910 | 2.477 | 0.2337 | 3.69084   | 0.05471 | 63    |

Since the genetic mode of inheritance is typically unknown, it might be beneficial to use the maximum over the gTDT statistics for an additive, a dominant, and a recessive effect as test statistic, which can be done using the function `colTDTmaxStat()`

```
> max.stat <- colTDTmaxStat(mat.test)
> max.stat
```

Maximum Genotypic TDT Statistic

Top 5 SNPs:

|      | Max-Statistic | Additive | Dominant | Recessive |
|------|---------------|----------|----------|-----------|
| SNP6 | 5.1295        | 3.6908   | 1.14571  | 5.12953   |
| SNP2 | 3.1569        | 0.9772   | 0.04811  | 3.15688   |
| SNP3 | 2.7150        | 1.0209   | 2.71503  | 0.76851   |
| SNP4 | 0.6990        | 0.3994   | 0.69897  | 0.01234   |
| SNP5 | 0.2156        | 0.1950   | 0.07337  | 0.21555   |

This function just computes the MAX gTDT statistic, i.e. the maximum over the three gTDT statistics, since in contrast to these gTDT statistics, which under the null hypothesis follow an asymptotic  $\chi^2_1$ -distribution, the null distribution of the MAX gTDT statistic

is unknown, and must therefore be estimated by a (time-consuming) permutation procedure. To also determine permutation-based p-values, `colTDTmaxTest()` can be applied to a matrix in genotype matrix. For example,

```
> max.out <- colTDTmaxTest(mat.test, perm=1000)
```

computes p-values for the six SNPs in `mat.test` based on 1000 permutations of the case-pseudo-control status.

```
> max.out
```

Maximum Genotypic TDT

Top 5 SNPs:

|      | Max-Statistic | Additive | Dominant | Recessive | p-Value |
|------|---------------|----------|----------|-----------|---------|
| SNP6 | 5.1295        | 3.6908   | 1.14571  | 5.12953   | 0.050   |
| SNP2 | 3.1569        | 0.9772   | 0.04811  | 3.15688   | 0.178   |
| SNP3 | 2.7150        | 1.0209   | 2.71503  | 0.76851   | 0.204   |
| SNP4 | 0.6990        | 0.3994   | 0.69897  | 0.01234   | 0.668   |
| SNP5 | 0.2156        | 0.1950   | 0.07337  | 0.21555   | 0.890   |

All two-way interactions comprised a matrix in genotype format can be tested using the function `colTDT2way()`. Since both the gTDT for two-way interactions and the likelihood ratio test of Cordell *et al.* (2004) assume that the two considered loci are unlinked, the testing might fail, i.e. the fitting of the conditional logistic regression might not work properly, if the two SNPs are in (strong) LD. (Another reason why the fitting might not work properly is that the minor allele frequencies of both SNPs are very small.) Therefore, `colTDT2way()` provides an argument called `genes` that allows specifying which SNP belongs to which LD-block, gene, or genetic region. If `genes` is not specified, the interactions between all  $m(m - 1)/2$  pairs of the  $m$  SNPs in a matrix are tested. If specified, only the interactions between SNPs showing different values of `genes` are tested.

If we thus assume that the first two SNPs in `mat.test` belong to gene G1 and the other four SNPs to G2

```
> genes <- paste("G", rep(1:2, c(2,4)), sep="")
> genes
```

```
[1] "G1" "G1" "G2" "G2" "G2" "G2"
```

then only the four interactions between SNP1 and each SNP from gene G2, as well as the four interactions between SNP2 and each SNP from gene G2 are tested, when calling

```
> tdt2.out <- colTDT2way(mat.test, genes=genes)
> tdt2.out
```

Genotypic TDT for Epistatic Interactions (Using 15 Pseudo Controls)

Top 5 SNP Interactions (Likelihood Ratio Test):

|             | LL (with IAs) | LL (w/o IAs) | Statistic | P-Value  | Genes   |
|-------------|---------------|--------------|-----------|----------|---------|
| SNP1 : SNP5 | -269.5        | -277.0       | 15.069    | 0.004561 | G1 : G2 |
| SNP2 : SNP4 | -270.3        | -275.0       | 9.528     | 0.049167 | G1 : G2 |
| SNP1 : SNP3 | -273.0        | -275.2       | 4.440     | 0.349724 | G1 : G2 |
| SNP2 : SNP5 | -273.3        | -275.3       | 3.871     | 0.423763 | G1 : G2 |
| SNP2 : SNP6 | -271.2        | -272.6       | 2.805     | 0.591008 | G1 : G2 |

Again, by default the top five SNP interactions are shown. The statistics for all eight interactions can be displayed by calling

```
> print(tdt2.out, 8)
```

Genotypic TDT for Epistatic Interactions (Using 15 Pseudo Controls)

Likelihood Ratio Test:

|             | LL (with IAs) | LL (w/o IAs) | Statistic | P-Value  | Genes   |
|-------------|---------------|--------------|-----------|----------|---------|
| SNP1 : SNP3 | -273.0        | -275.2       | 4.440     | 0.349724 | G1 : G2 |
| SNP1 : SNP4 | -275.9        | -276.8       | 1.653     | 0.799239 | G1 : G2 |
| SNP1 : SNP5 | -269.5        | -277.0       | 15.069    | 0.004561 | G1 : G2 |
| SNP1 : SNP6 | -273.3        | -274.3       | 2.050     | 0.726494 | G1 : G2 |
| SNP2 : SNP3 | -273.0        | -273.4       | 0.778     | 0.941371 | G1 : G2 |
| SNP2 : SNP4 | -270.3        | -275.0       | 9.528     | 0.049167 | G1 : G2 |
| SNP2 : SNP5 | -273.3        | -275.3       | 3.871     | 0.423763 | G1 : G2 |
| SNP2 : SNP6 | -271.2        | -272.6       | 2.805     | 0.591008 | G1 : G2 |

In genetic association studies, it is often also of interest to test gene-environment interactions, where most of the usually considered environmental variables are binary. The R package `trio` therefore also provides a function called `colGxE` to test the interactions between each of the SNPs comprised by a matrix in genotype format and a binary environmental variable with values zero and one. If we, for example, assume that the children in the first 50 trios comprised by (the first 150 rows of) `mat.test` are girls, and the remaining 50 are boys,

```
> sex <- rep(0:1, e=50)
```

then we can test the interactions between the six SNPs in `mat.test` and the environmental variable "sex" by

```
> gxe.out <- colGxE(mat.test, sex)
> gxe.out
```

Genotypic TDT for GxE Interactions with Binary E

Model Type: Additive

Top 5 GxE Interactions:

|      | Coef    | OR     | Lower  | Upper | SE     | Statistic | p-value | Trios0 | Trios1 |
|------|---------|--------|--------|-------|--------|-----------|---------|--------|--------|
| SNP2 | 0.5849  | 1.7949 | 0.8134 | 3.961 | 0.4038 | 2.0982    | 0.1475  | 37     | 36     |
| SNP1 | -0.4257 | 0.6533 | 0.2896 | 1.474 | 0.4151 | 1.0518    | 0.3051  | 32     | 40     |
| SNP6 | -0.3878 | 0.6786 | 0.2697 | 1.708 | 0.4708 | 0.6783    | 0.4102  | 34     | 29     |
| SNP4 | 0.2624  | 1.3000 | 0.5668 | 2.982 | 0.4235 | 0.3838    | 0.5356  | 36     | 35     |
| SNP5 | 0.2007  | 1.2222 | 0.5129 | 2.912 | 0.4430 | 0.2052    | 0.6506  | 33     | 31     |

Effects of the SNPs in the Corresponding GxE Models:

|      | Coef    | OR     | Lower  | Upper | SE     | Statistic | p-value |
|------|---------|--------|--------|-------|--------|-----------|---------|
| SNP2 | -0.5108 | 0.6000 | 0.3345 | 1.076 | 0.2981 | 2.9356    | 0.08665 |
| SNP1 | 0.1744  | 1.1905 | 0.6664 | 2.127 | 0.2960 | 0.3469    | 0.55585 |
| SNP6 | 0.6242  | 1.8667 | 0.9970 | 3.495 | 0.3200 | 3.8051    | 0.05110 |
| SNP4 | -0.2624 | 0.7692 | 0.4294 | 1.378 | 0.2974 | 0.7781    | 0.37771 |
| SNP5 | 0.0000  | 1.0000 | 0.5462 | 1.831 | 0.3086 | 0.0000    | 1.00000 |

In this situation, a conditional logistic regression model  $\beta_1 G + \beta_2 (G \times E)$  is fitted for each SNP, where  $G$  is a variable coding for an additive effect of the SNP, and  $G \times E$  is the corresponding gene-environment interaction. Analogously to the other gTDT functions, a dominant or a recessive effect can also be considered by changing the argument `model` of `colGxE`. The output contains the same statistics as, for example, `colTDT` for both  $\beta_1$  and  $\beta_2$ , where the statistics for  $\beta_2$  are printed first, as these are here the effects of interest. The printing of the statistics for the testing of  $G$  can be avoided by calling

```
> print(gxe.out, onlyGxE=TRUE)
```

Genotypic TDT for GxE Interactions with Binary E

Model Type: Additive

Top 5 GxE Interactions:

|      | Coef    | OR     | Lower  | Upper | SE     | Statistic | p-value | Trios0 | Trios1 |
|------|---------|--------|--------|-------|--------|-----------|---------|--------|--------|
| SNP2 | 0.5849  | 1.7949 | 0.8134 | 3.961 | 0.4038 | 2.0982    | 0.1475  | 37     | 36     |
| SNP1 | -0.4257 | 0.6533 | 0.2896 | 1.474 | 0.4151 | 1.0518    | 0.3051  | 32     | 40     |
| SNP6 | -0.3878 | 0.6786 | 0.2697 | 1.708 | 0.4708 | 0.6783    | 0.4102  | 34     | 29     |
| SNP4 | 0.2624  | 1.3000 | 0.5668 | 2.982 | 0.4235 | 0.3838    | 0.5356  | 36     | 35     |
| SNP5 | 0.2007  | 1.2222 | 0.5129 | 2.912 | 0.4430 | 0.2052    | 0.6506  | 33     | 31     |

## 4 Generating data for trio logic regression input

If interactions of a higher order than two are of interest, trio logic regression can be used to detect disease-associated SNP interactions of any order.

To generate data that can be used as input in trio logic regression, the sequential application of two functions is required. The function `trio.check()` evaluates whether or not Mendelian errors are present in the data (stored either in linkage or in genotype format, see Section 4.1). If no Mendelian inconsistencies are detected, this function creates an object that is passed to the function `trio()`. The latter function then generates a matrix of the genotype information for the affected probands and the inferred pseudo-controls, taking the observed LD structure into account. Missing data are imputed in the process. The user, however, has to supply the information for the lengths of the LD blocks. A function called `findLDblocks()` for identifying LD blocks, and thus, for specifying the length of the blocks is therefore also contained in this package (see Section 5). Given the lengths of the LD blocks, the haplotype frequencies can be estimated, using the function `haplo.em()` in the `haplo.stat` package.

### 4.1 Supported file formats and elementary data processing

In this section, we show how to generate data suitable for input to trio logic regression from complete pedigree data without Mendelian errors. The function `trio.check()` requires

that the trio data are already available as a data frame or matrix, either in linkage/ped format (the default), or in genotype format (for reading a ped file into R, see Section 3).

The first function used is always `trio.check()`. Unless otherwise specified, this function assumes that the data are in linkage format. If no Mendelian inconsistencies in the data provided are identified, `trio.check()` creates an object that can be processed in the subsequent analysis with this package. The genotype information for each SNP will be converted into a single variable, denoting the number of variant alleles.

If we thus would like to check whether the data frame `trio.ped1` contains Mendelian errors, we call

```
> data(trio.data)
> trio.tmp <- trio.check(dat=trio.ped1)
> str(trio.tmp, max=1)
```

List of 2

```
$ trio : 'data.frame':      300 obs. of  12 variables:
 $ errors: NULL
```

```
> trio.tmp$trio[1:6,]
```

|   | famid | pid | snp1 | snp2 | snp3 | snp4 | snp5 | snp6 | snp7 | snp8 | snp9 | snp10 |
|---|-------|-----|------|------|------|------|------|------|------|------|------|-------|
| 1 | 10001 | 1   | 0    | 0    | 1    | 1    | 1    | 1    | 0    | 0    | 0    | 0     |
| 2 | 10001 | 2   | 0    | 2    | 0    | 0    | 2    | 0    | 0    | 0    | 0    | 0     |
| 3 | 10001 | 3   | 0    | 1    | 1    | 1    | 1    | 1    | 0    | 0    | 0    | 0     |
| 4 | 10002 | 1   | 0    | 0    | 2    | 2    | 0    | 2    | 0    | 0    | 0    | 1     |
| 5 | 10002 | 2   | 0    | 2    | 0    | 0    | 2    | 0    | 0    | 0    | 0    | 0     |
| 6 | 10002 | 3   | 0    | 1    | 1    | 1    | 1    | 1    | 0    | 0    | 0    | 0     |

Taking the LD structure of the SNPs into account is imperative when creating the genotypes for the pseudo-controls. This requires information on the LD blocks. However, there are many ways to delineate this block structure, and in the absence of a consensus what the best approach is, researchers have different preferences, and thus, results can be different. In the function `findLDblocks()`, a modified version of the method of Gabriel *et al.* (2002) has been implemented, which can be used to specify the block structure by

```
> table(foundBlocks$blocks)
```

if `foundBlocks` is the output of `findLDblocks()` (for details, see Section 5).

The function `trio()`, which operates on an output object of `trio.check()`, accepts the block length information as an argument (in the following, we assume that the block structure is given by `c(1, 4, 2, 3)`, i.e. the first block consists only of the first SNP, the second block of the next four SNPs, the third of the following two SNPs, and the last block of the remaining three SNPs). If this argument is not specified, a uniform block length of 1 (i.e. no LD structure) is assumed. If the haplotype frequencies are not specified, they are estimated from the parents' genotypes (more information on this in the following sections). The function `trio()` then returns a list that contains the genotype information in binary format, suitable as input for trio logic regression: `bin` is a matrix with the conditional logistic regression response in the first columns, and each SNP as two binary variables using dominant and recessive coding. The list element `miss` contains information about missing values in the original data, and `freq` contains information on the estimated haplotype frequencies.

```
> trio.bin <- trio(trio.dat=trio.tmp, blocks=c(1,4,2,3))
> str(trio.bin, max=1)
```

```
List of 3
```

```
$ bin : num [1:400, 1:21] 3 0 0 0 3 0 0 0 3 0 ...
  ..- attr(*, "dimnames")=List of 2
$ miss: NULL
$ freq:'data.frame':      19 obs. of  3 variables:
```

```
> trio.bin$bin[1:8,]
```

|      | y      | snp1.D | snp1.R | snp2.D | snp2.R | snp3.D | snp3.R | snp4.D | snp4.R  | snp5.D  | snp5.R |
|------|--------|--------|--------|--------|--------|--------|--------|--------|---------|---------|--------|
| [1,] | 3      | 0      | 0      | 1      | 0      | 1      | 0      | 1      | 0       | 1       | 0      |
| [2,] | 0      | 0      | 0      | 1      | 0      | 0      | 0      | 0      | 0       | 1       | 1      |
| [3,] | 0      | 0      | 0      | 1      | 0      | 0      | 0      | 0      | 0       | 1       | 1      |
| [4,] | 0      | 0      | 0      | 1      | 0      | 1      | 0      | 1      | 0       | 1       | 0      |
| [5,] | 3      | 0      | 0      | 1      | 0      | 1      | 0      | 1      | 0       | 1       | 0      |
| [6,] | 0      | 0      | 0      | 1      | 0      | 1      | 0      | 1      | 0       | 1       | 0      |
| [7,] | 0      | 0      | 0      | 1      | 0      | 1      | 0      | 1      | 0       | 1       | 0      |
| [8,] | 0      | 0      | 0      | 1      | 0      | 1      | 0      | 1      | 0       | 1       | 0      |
|      | snp6.D | snp6.R | snp7.D | snp7.R | snp8.D | snp8.R | snp9.D | snp9.R | snp10.D | snp10.R |        |
| [1,] | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       | 0       |        |
| [2,] | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       | 0       |        |
| [3,] | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       | 0       |        |

```

[4,] 0 0 0 0 0 0 0 0 0 0 0
[5,] 1 0 0 0 0 0 0 0 0 0 0
[6,] 1 0 0 0 0 0 0 0 0 1 0
[7,] 1 0 0 0 0 0 0 0 0 1 0
[8,] 1 0 0 0 0 0 0 0 0 0 0

```

As mentioned above, the `trio` package also accommodates trio genotype data. The object `trio.gen1`, available in the R package, is an example of such a data set. Equivalent to `trio.ped1` used above, it contains information for 10 SNPs in 100 trios. When used in `trio.check()`, the argument `is.linkage` needs to be set to `FALSE`. The output from this function is then identical to the one shown derived from the linkage file, and can be passed to the function `trio()`.

```

> data(trio.data)
> str(trio.gen1)

'data.frame':      300 obs. of  12 variables:
 $ famid: int  10001 10001 10001 10002 10002 10002 10003 10003 10003 10004 ...
 $ pid  : int   1 2 3 1 2 3 1 2 3 1 ...
 $ snp1 : int   0 0 0 0 0 0 0 0 0 1 ...
 $ snp2 : int   0 2 1 0 2 1 0 0 0 0 ...
 $ snp3 : int   1 0 1 2 0 1 0 1 0 1 ...
 $ snp4 : int   1 0 1 2 0 1 0 1 0 1 ...
 $ snp5 : int   1 2 1 0 2 1 1 0 0 0 ...
 $ snp6 : int   1 0 1 2 0 1 0 1 0 1 ...
 $ snp7 : int   0 0 0 0 0 0 0 0 0 0 ...
 $ snp8 : int   0 0 0 0 0 0 0 0 0 0 ...
 $ snp9 : int   0 0 0 0 0 0 0 0 0 0 ...
 $ snp10: int   0 0 0 1 0 0 0 0 0 1 ...

> trio.gen1[1:10,1:12]

   famid pid snp1 snp2 snp3 snp4 snp5 snp6 snp7 snp8 snp9 snp10
1  10001   1    0    0    1    1    1    1    0    0    0    0
2  10001   2    0    2    0    0    2    0    0    0    0    0
3  10001   3    0    1    1    1    1    1    0    0    0    0
4  10002   1    0    0    2    2    0    2    0    0    0    1
5  10002   2    0    2    0    0    2    0    0    0    0    0
6  10002   3    0    1    1    1    1    1    0    0    0    0
7  10003   1    0    0    0    0    1    0    0    0    0    0
8  10003   2    0    0    1    1    0    1    0    0    0    0
9  10003   3    0    0    0    0    0    0    0    0    0    0
10 10004   1    1    0    1    1    0    1    0    0    0    1

> trio.tmp <- trio.check(dat=trio.gen1, is.linkage=F)
> trio.bin <- trio(trio.dat=trio.tmp, blocks=c(1,4,2,3))

```

## 4.2 Missing genotype information

Missing genotypes in `ped(igree)` files are typically encoded using the integer 0. The data files can be processed as before if they contain such missing values:

```
> data(trio.data)
> str(trio.ped2)

'data.frame':      300 obs. of  26 variables:
 $ famid   : int  10001 10001 10001 10002 10002 10002 10003 10003 10003 10004 ...
 $ pid     : int   1 2 3 1 2 3 1 2 3 1 ...
 $ fatid   : int   0 0 1 0 0 1 0 0 1 0 ...
 $ motid   : int   0 0 2 0 0 2 0 0 2 0 ...
 $ sex     : int   1 2 2 1 2 1 1 2 1 1 ...
 $ affected: int   0 0 2 0 0 2 0 0 2 0 ...
 $ snp1_1  : int   1 1 1 1 1 1 1 1 1 1 ...
 $ snp1_2  : int   1 1 1 1 1 1 1 1 1 2 ...
 $ snp2_1  : int   1 0 1 1 2 1 1 1 1 1 ...
 $ snp2_2  : int   1 0 2 1 2 2 1 1 1 1 ...
 $ snp3_1  : int   1 1 1 2 0 1 1 1 1 1 ...
 $ snp3_2  : int   2 1 2 2 0 2 1 2 1 2 ...
 $ snp4_1  : int   1 0 1 2 1 1 1 1 1 1 ...
 $ snp4_2  : int   2 0 2 2 1 2 1 2 1 2 ...
 $ snp5_1  : int   1 2 1 1 2 1 1 1 1 1 ...
 $ snp5_2  : int   2 2 2 1 2 2 2 1 1 1 ...
 $ snp6_1  : int   1 1 1 0 1 1 1 1 1 1 ...
 $ snp6_2  : int   2 1 2 0 1 2 1 2 1 2 ...
 $ snp7_1  : int   1 1 1 1 1 1 1 1 0 1 ...
 $ snp7_2  : int   1 1 1 1 1 1 1 1 0 1 ...
 $ snp8_1  : int   1 1 1 1 0 1 1 1 0 1 ...
 $ snp8_2  : int   1 1 1 1 0 1 1 1 0 1 ...
 $ snp9_1  : int   1 1 1 1 1 1 1 0 1 1 ...
 $ snp9_2  : int   1 1 1 1 1 1 1 0 1 1 ...
 $ snp10_1 : int   1 1 1 1 1 1 1 1 1 1 ...
 $ snp10_2 : int   1 1 1 2 1 1 1 1 1 2 ...

> trio.tmp <- trio.check(dat=trio.ped2)
> trio.tmp$trio[1:6,]

  famid pid snp1 snp2 snp3 snp4 snp5 snp6 snp7 snp8 snp9 snp10
1 10001  1    0    0    1    1    1    1    0    0    0    0
2 10001  2    0   NA    0   NA    2    0    0    0    0    0
3 10001  3    0    1    1    1    1    1    0    0    0    0
4 10002  1    0    0    2    2    0   NA    0    0    0    1
5 10002  2    0    2   NA    0    2    0    0   NA    0    0
6 10002  3    0    1    1    1    1    1    0    0    0    0
```

Since trio logic regression requires complete data, the function `trio()` also performs an imputation of the missing genotypes. The imputation is based on estimated haplotypes, using the block length information specified by the user. In a later section we demonstrate how this imputation can be run more efficiently when haplotype frequency estimates are already available.

```
> trio.bin <- trio(trio.dat=trio.tmp, blocks=c(1,4,2,3))
> trio.bin$bin[1:8,]
```

|      | y | snp1.D | snp1.R | snp2.D | snp2.R | snp3.D | snp3.R | snp4.D | snp4.R | snp5.D | snp5.R |
|------|---|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| [1,] | 3 | 0      | 0      | 1      | 0      | 1      | 0      | 1      | 0      | 1      | 0      |
| [2,] | 0 | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 1      | 1      |
| [3,] | 0 | 0      | 0      | 1      | 0      | 0      | 0      | 0      | 0      | 1      | 1      |
| [4,] | 0 | 0      | 0      | 0      | 0      | 1      | 0      | 1      | 0      | 1      | 0      |
| [5,] | 3 | 0      | 0      | 1      | 0      | 1      | 0      | 1      | 0      | 1      | 0      |
| [6,] | 0 | 0      | 0      | 1      | 0      | 1      | 0      | 1      | 0      | 1      | 0      |
| [7,] | 0 | 0      | 0      | 1      | 0      | 1      | 0      | 1      | 0      | 1      | 0      |
| [8,] | 0 | 0      | 0      | 1      | 0      | 1      | 0      | 1      | 0      | 1      | 0      |

|      | snp6.D | snp6.R | snp7.D | snp7.R | snp8.D | snp8.R | snp9.D | snp9.R | snp10.D | snp10.R |
|------|--------|--------|--------|--------|--------|--------|--------|--------|---------|---------|
| [1,] | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       | 0       |
| [2,] | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       | 0       |
| [3,] | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       | 0       |
| [4,] | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       | 0       |
| [5,] | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       | 0       |
| [6,] | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 1       | 0       |
| [7,] | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 1       | 0       |
| [8,] | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       | 0       |

Missing data in genotypes files should be encoded using NA, the conventional symbol in R to indicate missing values.

```
> data(trio.data)
> str(trio.gen2)
```

```
'data.frame':      300 obs. of  12 variables:
 $ famid: int  10001 10001 10001 10002 10002 10002 10003 10003 10003 10004 ...
 $ pid  : int  1 2 3 1 2 3 1 2 3 1 ...
 $ snp1 : int  0 0 0 0 0 0 0 0 0 1 ...
 $ snp2 : int  0 2 1 NA NA 1 0 0 0 0 ...
 $ snp3 : int  1 NA 1 2 0 1 0 NA 0 1 ...
 $ snp4 : int  1 0 1 NA 0 1 0 1 0 1 ...
 $ snp5 : int  1 2 1 0 2 1 1 NA 0 0 ...
 $ snp6 : int  1 0 1 NA 0 1 0 1 0 1 ...
 $ snp7 : int  0 0 0 0 0 0 0 0 0 0 ...
 $ snp8 : int  0 0 NA 0 0 0 0 NA 0 0 ...
```

```

$ snp9 : int  0 0 0 0 0 0 0 0 0 0 ...
$ snp10: int  0 0 0 1 0 0 0 0 0 1 ...

> trio.tmp <- trio.check(dat=trio.gen2, is.linkage=FALSE)
> trio.bin <- trio(trio.dat=trio.tmp, blocks=c(1,4,2,3))
> trio.bin$bin[1:8,]

      y snp1.D snp1.R snp2.D snp2.R snp3.D snp3.R snp4.D snp4.R snp5.D snp5.R
[1,] 3      0      0      1      0      1      0      1      0      1      0
[2,] 0      0      0      1      0      0      0      0      0      1      1
[3,] 0      0      0      1      0      0      0      0      0      1      1
[4,] 0      0      0      1      0      1      0      1      0      1      0
[5,] 3      0      0      1      0      1      0      1      0      1      0
[6,] 0      0      0      1      0      1      0      1      0      1      0
[7,] 0      0      0      0      0      1      0      1      0      1      0
[8,] 0      0      0      0      0      1      0      1      0      1      0
      snp6.D snp6.R snp7.D snp7.R snp8.D snp8.R snp9.D snp9.R snp10.D snp10.R
[1,]      1      0      0      0      0      0      0      0      0      0
[2,]      0      0      0      0      0      0      0      0      0      0
[3,]      1      0      0      0      0      0      0      0      0      0
[4,]      0      0      0      0      0      0      0      0      0      0
[5,]      1      0      0      0      0      0      0      0      0      0
[6,]      1      0      0      0      0      0      0      0      1      0
[7,]      0      0      0      0      0      0      0      0      0      0
[8,]      0      0      0      0      0      0      0      0      1      0

```

As the user might also be interested in the completed genotype data in the original format (genotype or linkage file), the function `trio()` also allows for this option by using the argument `logic = FALSE`. In the resulting object, the matrix `bin` is then replaced by the data frame `trio`, and `miss` and `freq` are also returned.

```

> data(trio.data)
> trio.tmp <- trio.check(dat=trio.gen2, is.linkage=FALSE)
> trio.imp <- trio(trio.dat=trio.tmp, blocks=c(1,4,2,3), logic=FALSE)
> str(trio.imp, max=1)

```

```

List of 3
 $ trio:'data.frame':      300 obs. of  12 variables:
 $ miss:'data.frame':      250 obs. of  5 variables:
 $ freq:'data.frame':      19 obs. of  3 variables:

```

```

> trio.imp$miss[c(1:6),]

```

```

      famid pid snp r  c
1 10001    2   3 2  5
2 10001    3   8 3 10
3 10002    1   2 4  4

```

```

4 10002  1  4 4  6
5 10002  1  6 4  8
6 10002  2  2 5  4

```

```
> print(trio.gen2[1:6,])
```

|   | famid | pid | snp1 | snp2 | snp3 | snp4 | snp5 | snp6 | snp7 | snp8 | snp9 | snp10 |
|---|-------|-----|------|------|------|------|------|------|------|------|------|-------|
| 1 | 10001 | 1   | 0    | 0    | 1    | 1    | 1    | 1    | 0    | 0    | 0    | 0     |
| 2 | 10001 | 2   | 0    | 2    | NA   | 0    | 2    | 0    | 0    | 0    | 0    | 0     |
| 3 | 10001 | 3   | 0    | 1    | 1    | 1    | 1    | 1    | 0    | NA   | 0    | 0     |
| 4 | 10002 | 1   | 0    | NA   | 2    | NA   | 0    | NA   | 0    | 0    | 0    | 1     |
| 5 | 10002 | 2   | 0    | NA   | 0    | 0    | 2    | 0    | 0    | 0    | 0    | 0     |
| 6 | 10002 | 3   | 0    | 1    | 1    | 1    | 1    | 1    | 0    | 0    | 0    | 0     |

```
> print(trio.imp$trio[1:6,])
```

|   | famid | pid | snp1 | snp2 | snp3 | snp4 | snp5 | snp6 | snp7 | snp8 | snp9 | snp10 |
|---|-------|-----|------|------|------|------|------|------|------|------|------|-------|
| 1 | 10001 | 1   | 0    | 0    | 1    | 1    | 1    | 1    | 0    | 0    | 0    | 0     |
| 2 | 10001 | 2   | 0    | 2    | 0    | 0    | 2    | 0    | 0    | 0    | 0    | 0     |
| 3 | 10001 | 3   | 0    | 1    | 1    | 1    | 1    | 1    | 0    | 0    | 0    | 0     |
| 4 | 10002 | 1   | 0    | 0    | 2    | 2    | 0    | 2    | 0    | 0    | 0    | 1     |
| 5 | 10002 | 2   | 0    | 1    | 0    | 0    | 2    | 0    | 0    | 0    | 0    | 0     |
| 6 | 10002 | 3   | 0    | 1    | 1    | 1    | 1    | 1    | 0    | 0    | 0    | 0     |

The same applies to pedigree data:

```

> data(trio.data)
> trio.tmp <- trio.check(dat=trio.ped2)
> trio.imp <- trio(trio.dat=trio.tmp, blocks=c(1,4,2,3), logic=FALSE)

```

## 4.3 Mendelian errors

To delineate the genotype information for the pseudo-controls, the trio data must not contain any Mendelian errors. The function `trio.check()` returns a warning, and an R object with relevant information when Mendelian errors are encountered is created.

```

> data(trio.data)
> trio.tmp <- trio.check(dat=trio.ped.err)

[1] "Found Mendelian error(s)."

> str(trio.tmp, max=1)

```

```

List of 3
 $ trio      : NULL
 $ errors    : 'data.frame':      4 obs. of  5 variables:
 $ trio.err:'data.frame':     300 obs. of 12 variables:

> trio.tmp$errors

   trio famid snp r  c
1    1 10001   9 1 11
2    1 10001  10 1 12
3    2 10002  10 4 12
4    3 10003  10 7 12

```

In this data set, trio 1, for example, contains two Mendelian errors, in SNPs 9 and 10.

```

> trio.tmp$trio.err[1:3, c(1,2, 11:12)]

   famid pid snp9 snp10
1 10001   1    0     1
2 10001   2    0     2
3 10001   3    2     0

> trio.ped.err[1:3,c(1:2, 23:26)]

   famid pid snp9_1 snp9_2 snp10_1 snp10_2
1 10001   1     1     1         1         2
2 10001   2     1     1         2         2
3 10001   3     2     2         1         1

```

It is the user's responsibility to find the cause for the Mendelian errors and correct those, if possible. However, Mendelian inconsistencies are often due to genotyping errors and thus, it might not be possible to correct those in a very straight-forward manner. In this instance, the user might want to encode the genotypes that cause theses Mendelian errors in some of the trios as missing data. The argument `replace = TRUE` in `trio.check()` allows for this possibility. The resulting missing data can then be imputed as described in the previous section.

```

> trio.rep <- trio.check(dat=trio.ped.err, replace=TRUE)
> str(trio.rep, max=1)

```

```

List of 2
 $ trio : 'data.frame':      300 obs. of 12 variables:
 $ errors: NULL

```

```
> trio.rep$trio[1:3,11:12]
```

```
      snp9 snp10
1      NA      NA
2      NA      NA
3      NA      NA
```

The same option is available for data in genotype format with Mendelian inconsistencies.

```
> data(trio.data)
```

```
> trio.tmp <- trio.check(dat=trio.gen.err, is.linkage=FALSE)
```

```
[1] "Found Mendelian error(s)."
```

```
> trio.tmp$errors
```

```
      trio famid snp r c
1       1   2001   5 1 7
2       2   2002   5 4 7
```

```
> trio.tmp$trio.err[1:6, c(1,2,7), drop=F]
```

```
      famid pid snp5
6    2001   1     0
7    2001   2     0
5    2001   3     1
9    2002   1     1
10   2002   2     0
8    2002   3     2
```

```
> trio.rep <- trio.check(dat=trio.gen.err, is.linkage=FALSE, replace=TRUE)
```

```
> trio.rep$trio[1:6,c(1,2,7)]
```

```
      famid pid snp5
6    2001   1    NA
7    2001   2    NA
5    2001   3    NA
9    2002   1    NA
10   2002   2    NA
8    2002   3    NA
```

## 4.4 Using haplotype frequencies

As mentioned above, when estimates for the haplotype frequencies are already available, they can be used in the imputation of missing data and the delineation of the pseudo-controls. In case there are blocks of length one, i.e. SNPs not belonging to any LD

blocks, the minor allele frequencies of those SNPs are supplied. In this case, no haplotype estimation is required when the function `trio()` is run, which can result in substantial time savings.

As an example for the format of a file containing haplotype frequency estimates and SNP minor allele frequencies, the object `freq.hap` is available in the R package:

```
> data(trio.data)
> str(freq.hap)

'data.frame':      20 obs. of  3 variables:
 $ key : int  1 1 2 2 2 2 2 2 2 3 ...
 $ hap : int  1 2 1111 1112 1121 1221 1222 2112 2222 11 ...
 $ freq: num  0.8 0.2 0.3373 0.2059 0.0024 ...

> freq.hap[1:6,]

  key hap      freq
1   1   1 0.800000000
2   1   2 0.200000000
3   2 1111 0.337339745
4   2 1112 0.205929486
5   2 1121 0.002403846
6   2 1221 0.368589742
```

We can now impute the missing genotypes using these underlying haplotype frequencies.

```
> data(trio.data)
> trio.tmp <- trio.check(dat=trio.gen2, is.linkage=FALSE)
> trio.imp <- trio(trio.dat=trio.tmp, freq=freq.hap, logic=FALSE)
> str(trio.imp, max=1)
```

```
List of 3
 $ trio:'data.frame':      300 obs. of  12 variables:
 $ miss:'data.frame':      250 obs. of  5 variables:
 $ freq:'data.frame':      20 obs. of  3 variables:
```

```
> print(trio.gen2[1:6,])

  famid pid snp1 snp2 snp3 snp4 snp5 snp6 snp7 snp8 snp9 snp10
1 10001   1   0   0   1   1   1   1   0   0   0   0
2 10001   2   0   2  NA   0   2   0   0   0   0   0
3 10001   3   0   1   1   1   1   1   0  NA   0   0
4 10002   1   0  NA   2  NA   0  NA   0   0   0   1
5 10002   2   0  NA   0   0   2   0   0   0   0   0
6 10002   3   0   1   1   1   1   1   0   0   0   0
```

```
> print(trio.imp$trio[1:6,])
```

|   | famid | pid | snp1 | snp2 | snp3 | snp4 | snp5 | snp6 | snp7 | snp8 | snp9 | snp10 |
|---|-------|-----|------|------|------|------|------|------|------|------|------|-------|
| 1 | 10001 | 1   | 0    | 0    | 1    | 1    | 1    | 1    | 0    | 0    | 0    | 0     |
| 2 | 10001 | 2   | 0    | 2    | 0    | 0    | 2    | 0    | 0    | 0    | 0    | 0     |
| 3 | 10001 | 3   | 0    | 1    | 1    | 1    | 1    | 1    | 0    | 0    | 0    | 0     |
| 4 | 10002 | 1   | 0    | 0    | 2    | 2    | 0    | 1    | 0    | 0    | 0    | 1     |
| 5 | 10002 | 2   | 0    | 2    | 0    | 0    | 2    | 0    | 0    | 0    | 0    | 0     |
| 6 | 10002 | 3   | 0    | 1    | 1    | 1    | 1    | 1    | 0    | 0    | 0    | 0     |

## 4.5 Trio Logic Regression

Trio logic regression can be applied to case-parent trio data using the standard R function `logreg` (available in the R package `LogicReg`) to perform a logic regression by employing a plug-in provided by the R package `trio`. However, this requires to modify and recompile `LogicReg`, which can be done in the following way:

1. Download the package source for `LogicReg` from <http://cran.r-project.org/web/packages/LogicReg/index.html>.
2. Extract this package.
3. Replace the file `My_own_scoring.f` available in the subdirectory `src` of `LogicReg` by the file `My_own_scoring.f` provided in the subdirectory `plugin` of the R package `trio`.
4. Recompile the modified `LogicReg` by applying R CMD `build` to it (for details on how to check and build R packages, see <http://cran.r-project.org/doc/manuals/R-exts.html>).
5. Install this recompiled package.

After installing and loading this recompiled package, trio logic regression can be applied to `trio.bin` or to `trio.imp` (see Sections 4.2 and 4.3) by

```

> library(LogicReg)
> resp <- trio.bin$bin[,1]
> bin <- trio.bin$bin[,-1]
> triolr.out <- logreg(resp=resp, bin=bin, type=0, ntrees=1, ...)

```

where the ... should indicate that all other arguments of `logreg` such as `select` (type of model selection) and `nleaves` (the maximum number of leaves that the logic tree in the trio logic regression model is allowed to have) need to be or can be specified as in a typical logic regression analysis. One restriction is that in trio logic regression currently just one logic tree can be grown, and therefore, `ntrees` needs to be set to 1.

Adding the trio logic regression plug-in to `LogicReg` allows making use of almost all the features implemented in `logreg`. The only exceptions are the model selection procedures provided by `logreg`. The standard implementation cannot be applied to case-parent trio data, as the special structure of these data must be taken into account. Therefore, the R package `trio` provides special permutation tests via a function called `trio.permTest` that can be used to apply a null-model test with, for example, 20 permutations to the case-parent trio data in `trio.bin` by calling

```

> trio.permTest(triolr.out, n.perm=20)

```

and a conditional permutation test can be performed by

```

> trio.permTest(triolr.out, conditional=TRUE, n.perm=20)

```

## 5 Detection of LD blocks

For the estimation of the haplotype structure that might be used in the R function `trio()`, this package also includes functions for the fast computation of the pairwise  $D'$  and  $r^2$

values for hundreds or thousands of SNPs, and for the identification of LD blocks in these genotype data using a modified version of the algorithm proposed by Gabriel *et al.* (2002). For the latter, it is assumed that the SNPs are ordered by their position on the chromosomes.

These functions are not restricted to trio data, but can also be applied to population-based data. The only argument of these functions specifically included for trio data is `parentsOnly`. If set to `TRUE`, only the genotypes of the parents are used in the determination of the pairwise values of the LD measures and the estimation of the LD blocks. Furthermore, each parent is only considered once so that parents with more than one offspring do not bias the estimations. If trio data is used as input, the functions assume that the matrix containing the SNP data is in genotype format.

Here, we consider a simulated matrix `LDdata` from a population-based study. Thus, all subjects are assumed to be unrelated. This matrix contains simulated genotype data for 10 LD blocks each consisting of 5 SNPs each typed on 500 subjects. The pairwise  $D'$  and  $r^2$  values for the SNPs in this matrix can be computed by

```
> data(trio.data)
> ld.out <- getLD(LDdata, asMatrix=TRUE)
```

where by the default these values are stored in vectors to save memory. If `asMatrix` is set to `TRUE`, the values will be stored in matrices. The pairwise LD values for the first 10 SNPs (rounded to the second digit) can be displayed by

```
> round(ld.out$Dprime[1:10,1:10], 2)
```

|     | S1   | S2   | S3   | S4   | S5   | S6   | S7   | S8   | S9 | S10 |
|-----|------|------|------|------|------|------|------|------|----|-----|
| S1  | NA   | NA   | NA   | NA   | NA   | NA   | NA   | NA   | NA | NA  |
| S2  | 0.99 | NA   | NA   | NA   | NA   | NA   | NA   | NA   | NA | NA  |
| S3  | 0.98 | 1.00 | NA   | NA   | NA   | NA   | NA   | NA   | NA | NA  |
| S4  | 0.98 | 0.99 | 1.00 | NA   | NA   | NA   | NA   | NA   | NA | NA  |
| S5  | 0.97 | 0.98 | 0.99 | 1.00 | NA   | NA   | NA   | NA   | NA | NA  |
| S6  | 0.09 | 0.06 | 0.05 | 0.05 | 0.04 | NA   | NA   | NA   | NA | NA  |
| S7  | 0.11 | 0.09 | 0.08 | 0.08 | 0.07 | 0.99 | NA   | NA   | NA | NA  |
| S8  | 0.13 | 0.11 | 0.10 | 0.10 | 0.09 | 0.99 | 1.00 | NA   | NA | NA  |
| S9  | 0.14 | 0.11 | 0.10 | 0.11 | 0.10 | 0.99 | 1.00 | 1.00 | NA | NA  |
| S10 | 0.16 | 0.13 | 0.11 | 0.12 | 0.11 | 0.97 | 0.98 | 0.98 | 1  | NA  |

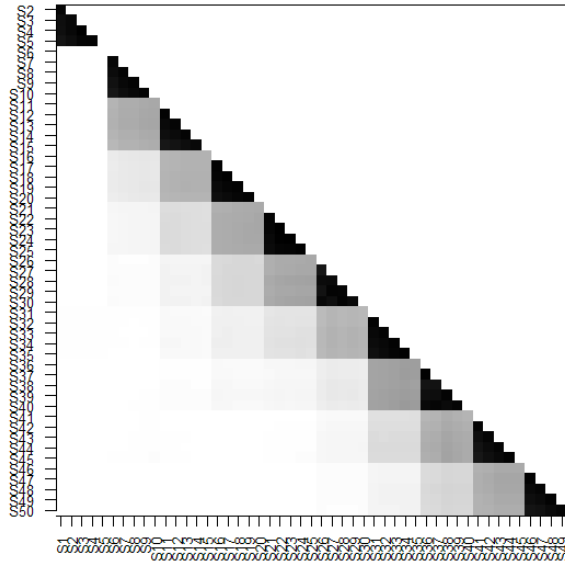


Figure 1: Pairwise  $r^2$  values for the SNPs from LDdata.

```
> round(ld.out$rSquare[1:10,1:10], 2)
```

|     | S1   | S2   | S3   | S4   | S5 | S6   | S7   | S8   | S9   | S10 |
|-----|------|------|------|------|----|------|------|------|------|-----|
| S1  | NA   | NA   | NA   | NA   | NA | NA   | NA   | NA   | NA   | NA  |
| S2  | 0.97 | NA   | NA   | NA   | NA | NA   | NA   | NA   | NA   | NA  |
| S3  | 0.94 | 0.97 | NA   | NA   | NA | NA   | NA   | NA   | NA   | NA  |
| S4  | 0.93 | 0.96 | 1.00 | NA   | NA | NA   | NA   | NA   | NA   | NA  |
| S5  | 0.91 | 0.94 | 0.98 | 0.98 | NA | NA   | NA   | NA   | NA   | NA  |
| S6  | 0.00 | 0.00 | 0.00 | 0.00 | 0  | NA   | NA   | NA   | NA   | NA  |
| S7  | 0.00 | 0.00 | 0.00 | 0.00 | 0  | 0.97 | NA   | NA   | NA   | NA  |
| S8  | 0.00 | 0.00 | 0.00 | 0.00 | 0  | 0.95 | 0.98 | NA   | NA   | NA  |
| S9  | 0.00 | 0.00 | 0.00 | 0.00 | 0  | 0.93 | 0.96 | 0.98 | NA   | NA  |
| S10 | 0.00 | 0.00 | 0.00 | 0.00 | 0  | 0.91 | 0.94 | 0.96 | 0.98 | NA  |

and the pairwise LD plot for all SNPs can be generated by

```
> plot(ld.out)
```

(see Figure 1). This figure shows the  $r^2$ -values. The  $D'$  values can be plotted by

```
> plot(ld.out, "Dprime")
```

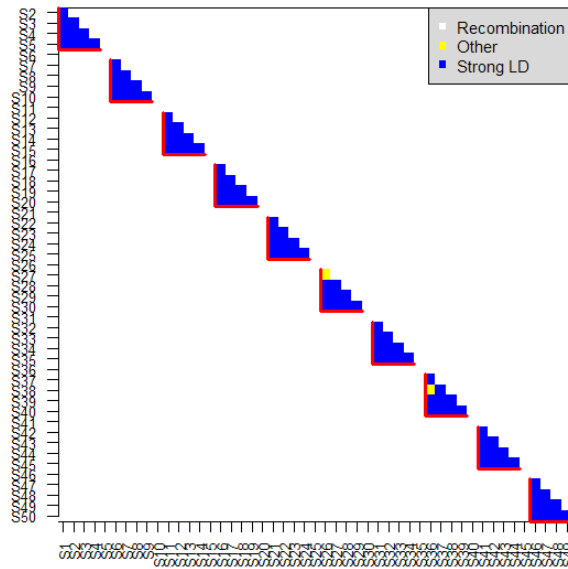


Figure 2: LD blocks as found by the modified algorithm of Gabriel *et al.* (2002). The borders of the LD blocks are marked by red lines. The color for the LD between each pair of SNPs is defined by the three categories used by Gabriel *et al.* (2002) to define the LD blocks.

(not shown).

The LD blocks in genotype data can be identified using the modified algorithm of Gabriel *et al.* (2002) by calling

```
> blocks <- findLDblocks(LDdata)
> blocks

Found 10 LD blocks containing between 5 and 5 SNPs.
0 of the 50 SNPs do not belong to a LD block.

Used Parameter:
Strong LD:      C_L >= 0.7 and C_U >= 0.98
Recombination: C_U < 0.9
(C_L and C_U are the lower and upper bound of
the 90%-confidence intervals for D')
LD blocks: Ratio >= 9
```

Alternatively, the output of `getLD()` can be used when `addVarN` has been set to `TRUE` in `getLD()` to store additional information on the pairwise LD values.

```
> ld.out2 <- getLD(LDdata, addVarN=TRUE)
```

```

> blocks2 <- findLDblocks(ld.out2)
> blocks2

Found 10 LD blocks containing between 5 and 5 SNPs.
0 of the 50 SNPs do not belong to a LD block.

Used Parameter:
Strong LD:      C_L >= 0.7 and C_U >= 0.98
Recombination: C_U < 0.9
(C_L and C_U are the lower and upper bound of
the 90%-confidence intervals for D')
LD blocks: Ratio >= 9

```

The blocks can also be plotted by

```

> plot(blocks)

```

(see Figure 2). In this figure, the borders of the LD blocks are marked by red lines. By default, the three categories used by the algorithm of Gabriel *et al.* (2002) to define the LD blocks are displayed. Since this algorithm is based on the  $D'$  values, it is also possible to show these values in the LD (block) plot.

```

> plot(blocks, "Dprime")

```

(see Figure 3).

As mentioned in Section 4, the haplotype structure required by `trio()` can be obtained by

```

> hap <- as.vector(table(blocks$blocks))
> hap

[1] 5 5 5 5 5 5 5 5 5 5

```

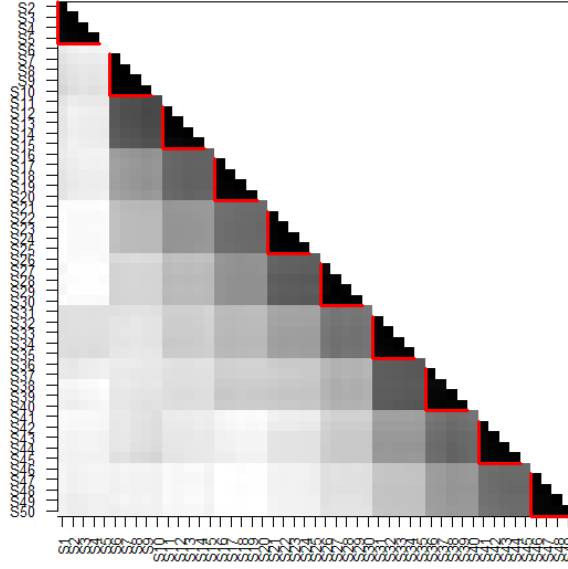


Figure 3: LD blocks as found by the modified algorithm of Gabriel *et al.* (2002). The borders of the LD blocks are marked by red lines. The darker the field for each pair of SNPs, the larger is the  $D'$  value for the corresponding SNP pair.

## 6 Simulation

The function `trio.sim()` simulates case-parents trio data when the disease risk of children is specified by (possibly higher-order) SNP interactions. The mating tables and the respective sampling probabilities depend on the haplotype frequencies (or SNP minor allele frequencies when the SNP does not belong to a block). This information is specified in the `freq` argument of the function `trio.sim()`. The probability of disease is assumed to be described by the logistic term  $\text{logit}(p) = \alpha + \beta \times \text{Interaction}$ , where  $\alpha = \text{logit}(\text{prev}) = \log\left(\frac{\text{prev}}{1-\text{prev}}\right)$  and  $\beta = \log(\text{OR})$ . The arguments `interaction`, `prev` and `OR`, are specified in the function `trio.sim()`. Generating the mating tables and the respective sampling probabilities, in particular for higher order interactions, can be very CPU and memory intensive. We show how this information, once it has been generated, can be used for future simulations, and thus, speed up the simulations dramatically.

## 6.1 A basic example

We use the built-in object `simuBkMap` in a basic example to show how to simulate case-parent trios when the disease risk depends on (possibly higher order) SNP interactions. This file contains haplotype frequency information on 15 blocks with a total of 45 loci. In this example, we specify that the children with two variant alleles on SNP1 and two variant alleles on SNP5 have a higher disease risk. We assume that  $\text{prev} = 0.001$  and  $\text{OR} = 2$  in the logistic model specifying disease risk, and simulate a single replicate of 20 trios total.

```
> data(trio.data)
> str(simuBkMap)

'data.frame':      66 obs. of  3 variables:
 $ key : Factor w/ 15 levels "10-1","10-10",...: 1 1 1 8 8 8 8 9 9 9 ...
 $ hap : int   11 21 22 121 122 111 222 21 22 12 ...
 $ freq: num   0.099 0.228 0.673 0.006 0.026 0.1 0.867 0.079 0.441 0.48 ...

> simuBkMap[1:7,]

   key hap  freq
1 10-1  11 0.099
2 10-1  21 0.228
3 10-1  22 0.673
4 10-2 121 0.006
5 10-2 122 0.026
6 10-2 111 0.100
7 10-2 222 0.867

> sim <- trio.sim(freq=simuBkMap, interaction="1R and 5R", prev=.001, OR=2,
+ n=20, rep=1)
> str(sim)

List of 1
 $ : num [1:60, 1:47] 1 1 1 2 2 2 3 3 3 4 ...
 ..- attr(*, "dimnames")=List of 2
 .. ..$ : NULL
 .. ..$ : chr [1:47] "famid" "pid" "snp1" "snp2" ...

> sim[[1]][1:6, 1:12]

      famid pid snp1 snp2 snp3 snp4 snp5 snp6 snp7 snp8 snp9 snp10
[1,]      1   1    2    2    2    2    2    1    2    2    2     0
[2,]      1   2    2    1    2    2    2    2    2    2    1     1
```

```
[3,] 1 3 2 2 2 2 2 2 2 2 1 1
[4,] 2 1 2 1 1 1 1 1 2 1 1 2
[5,] 2 2 2 2 1 1 1 1 1 1 1 1
[6,] 2 3 2 2 2 2 2 1 2 0 0 2
```

## 6.2 Using estimated haplotype frequencies

In this example we estimate the haplotype frequencies in the built-in data set `trio.gen1`, which contains genotypes for 10 SNPs in 100 trios. These estimated frequencies are then used to simulate 20 trios for the above specified disease risk model.

```
> data(trio.data)
> trio.tmp <- trio.check(dat=trio.gen1, is.linkage=FALSE)
> trio.impu <- trio(trio.dat=trio.tmp, blocks=c(1,4,2,3), logic=TRUE)
> str(trio.impu, max=2)

List of 3
 $ bin : num [1:400, 1:21] 3 0 0 0 3 0 0 0 3 0 ...
  ..- attr(*, "dimnames")=List of 2
 $ miss: NULL
 $ freq:'data.frame':      19 obs. of  3 variables:
  ..$ key :Class 'AsIs' chr [1:19] "ch-1" "ch-1" "ch-h2" "ch-h2" ...
  ..$ hap : num [1:19] 1 2 1111 1112 1121 ...
  ..$ freq: num [1:19] 0.9425 0.0575 0.325 0.2225 0.0075 ...

> trio.impu$freq[1:7,]

      key hap      freq
1  ch-1    1 9.42500e-01
2  ch-1    2 5.75000e-02
3 ch-h2 1111 3.25000e-01
4 ch-h2 1112 2.22500e-01
5 ch-h2 1121 7.50000e-03
6 ch-h2 1221 3.35000e-01
7 ch-h2 1222 3.47581e-09

> sim <- trio.sim(freq=trio.impu$freq, interaction="1R and 5R", prev=.001, OR=2,
+ n=20, rep=1)
> str(sim)

List of 1
 $ : num [1:60, 1:12] 1 1 1 2 2 2 3 3 3 4 ...
  ..- attr(*, "dimnames")=List of 2
  .. ..$ : NULL
  .. ..$ : chr [1:12] "famid" "pid" "snp1" "snp2" ...
```

```
> sim[[1]][1:6, ]
```

|      | famid | pid | snp1 | snp2 | snp3 | snp4 | snp5 | snp6 | snp7 | snp8 | snp9 | snp10 |
|------|-------|-----|------|------|------|------|------|------|------|------|------|-------|
| [1,] | 1     | 1   | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 1    | 0     |
| [2,] | 1     | 2   | 0    | 0    | 2    | 2    | 0    | 1    | 0    | 0    | 0    | 1     |
| [3,] | 1     | 3   | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 1     |
| [4,] | 2     | 1   | 1    | 0    | 1    | 1    | 1    | 0    | 0    | 0    | 0    | 0     |
| [5,] | 2     | 2   | 0    | 0    | 1    | 1    | 1    | 2    | 0    | 0    | 0    | 1     |
| [6,] | 2     | 3   | 0    | 0    | 1    | 1    | 1    | 1    | 0    | 0    | 0    | 0     |

As before, the object containing the haplotype frequency information can also be generated from external haplotype frequencies and SNP minor allele frequencies. In the following example we specify the haplotype frequencies, and generate two replicates of ten trios each.

```
> data(trio.data)
> sim <- trio.sim(freq=freq.hap, interaction="1R or 4D", prev=.001, OR=2,
+ n=10, rep=2)
> str(sim)
```

```
List of 2
 $ : num [1:30, 1:12] 1 1 1 2 2 2 3 3 3 4 ...
  ..- attr(*, "dimnames")=List of 2
  .. ..$ : NULL
  .. ..$ : chr [1:12] "famid" "pid" "snp1" "snp2" ...
 $ : num [1:30, 1:12] 1 1 1 2 2 2 3 3 3 4 ...
  ..- attr(*, "dimnames")=List of 2
  .. ..$ : NULL
  .. ..$ : chr [1:12] "famid" "pid" "snp1" "snp2" ...
```

```
> sim[[1]][1:6,]
```

|      | famid | pid | snp1 | snp2 | snp3 | snp4 | snp5 | snp6 | snp7 | snp8 | snp9 | snp10 |
|------|-------|-----|------|------|------|------|------|------|------|------|------|-------|
| [1,] | 1     | 1   | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 1     |
| [2,] | 1     | 2   | 0    | 1    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 1     |
| [3,] | 1     | 3   | 0    | 1    | 0    | 0    | 2    | 0    | 0    | 0    | 0    | 1     |
| [4,] | 2     | 1   | 0    | 0    | 0    | 0    | 0    | 2    | 0    | 0    | 0    | 0     |
| [5,] | 2     | 2   | 0    | 0    | 1    | 1    | 0    | 1    | 0    | 0    | 0    | 1     |
| [6,] | 2     | 3   | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0     |

### 6.3 Using step-stones

Generating the mating tables and the respective sampling probabilities necessary to simulate case-parent trios can be very time consuming for interaction models involving three or

more SNPs. In simulation studies, many replicates of similar data are usually required, and generating these sampling probabilities in each instance would be a large and avoidable computational burden (CPU and memory). The sampling probabilities depend foremost on the interaction term and the underlying haplotype frequencies, and as long as these remain constant in the simulation study, the mating table information and the sampling probabilities can be “recycled.” This is done by storing the relevant information (denoted as “step-stone”) as a binary R file in the working directory, and loading the binary file again in future simulations, speeding up the simulation process dramatically. It is even possible to change the parameters `prev` and `OR` in these additional simulations, as the sampling probabilities can be adjusted accordingly.

In the following example, we first simulate case-parent trios using a three-SNP interaction risk model, and save the step-stone object. We then simulate additional trios with a different parameter `OR`, using the previously generated information.

```
> data(trio.data)
> sim <- trio.sim(freq=freq.hap, interaction="1R or (6R and 10D)", prev=.001,
+ OR=2, n=10, rep=1)
> str(sim)
```

```
List of 1
 $ : num [1:30, 1:12] 1 1 1 2 2 2 3 3 3 4 ...
  .. attr(*, "dimnames")=List of 2
  .. ..$ : NULL
  .. ..$ : chr [1:12] "famid" "pid" "snp1" "snp2" ...
```

```
> sim[[1]][1:6,]
```

|      | famid | pid | snp1 | snp2 | snp3 | snp4 | snp5 | snp6 | snp7 | snp8 | snp9 | snp10 |
|------|-------|-----|------|------|------|------|------|------|------|------|------|-------|
| [1,] | 1     | 1   | 0    | 1    | 0    | 0    | 1    | 1    | 0    | 1    | 1    | 0     |
| [2,] | 1     | 2   | 1    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0     |
| [3,] | 1     | 3   | 1    | 1    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0     |
| [4,] | 2     | 1   | 0    | 0    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 0     |
| [5,] | 2     | 2   | 0    | 0    | 2    | 2    | 0    | 1    | 0    | 0    | 0    | 1     |
| [6,] | 2     | 3   | 0    | 0    | 1    | 1    | 1    | 1    | 1    | 0    | 0    | 1     |

```
> sim <- trio.sim(freq=freq.hap, interaction="1R or (6R and 10D)", prev=.001,
+ OR=3, n=10, rep=1, step.save="step3way")
> str(sim, max=1)
```

```

List of 1
 $ : num [1:30, 1:12] 1 1 1 2 2 2 3 3 3 4 ...
  ..- attr(*, "dimnames")=List of 2

> sim[[1]][1:6,]

      famid pid snp1 snp2 snp3 snp4 snp5 snp6 snp7 snp8 snp9 snp10
[1,]      1   1    1    0    1    1    0    0    0    0    0    0
[2,]      1   2    0    0    1    1    1    0    0    0    0    1
[3,]      1   3    1    0    1    1    0    0    0    0    0    1
[4,]      2   1    1    0    2    2    0    0    0    0    0    2
[5,]      2   2    0    0    2    2    0    0    0    0    0    1
[6,]      2   3    0    0    2    2    0    0    0    0    0    2

```

## Acknowledgments

Support was provided by NIH grants R01 DK061662 and R01 HL090577, and by DFG grant SCHW 1508/1-1 and SCHW 1508/2-1.

## References

- Cordell, H.J. (2002). Epistasis: What it means, what it doesn't mean, and statistical methods to detect it in humans. *Hum. Mol. Genet.*, **11**, 2463–2468.
- Cordell, H.J., Barratt, B.J., and Clayton, D.G. (2004). Case/pseudocontrol analysis in genetic association studies: A unified framework for detection of genotype and haplotype associations, gene-gene and gene-environment interactions, and parent-of-origin effects. *Genet. Epidemiol.*, **26**, 167–185.
- Gabriel, S.B., Schaffner, S.F., Nguyen, H., Moore, J.M., Roy, J., Blumenstiel, B., Higgins, J., DeFelice, M., Lochner, A., Faggart, M., Liu-Cordero, S.N., Rotimi, C., Adeyemo, A., Cooper, R., Ward, R., Lander, E.S., Daly, M.J., and Altshuler, D. (2002). The structure of haplotype blocks in the human genome. *Science*, **296**, 2225–2229.

- Li, Q., Fallin, M.D., Louis, T.A., Lasseter, V.K., McGrath, J.A., Avramopoulos, D., Wolyniec, P.S., Valle, D., Liang, K.Y., Pulver, A.E., and Ruczinski, I. (2010). Detection of SNP-SNP interactions in trios of parents with schizophrenic children. *Genet. Epidemiol.*, **34**, 396–406.
- Schaid, D.J. (1996). General score tests for associations of genetic markers with disease using cases and their parents. *Genet. Epidemiol.*, **13**, 423–449.