

# Preparing Case-Parent Trio Data and Detecting Disease-Associated SNPs, SNP Interactions, and Gene-Environment Interactions with `trio`/`trioClasses`

Holger Schwender, Qing Li, and Ingo Ruczinski

## 1 Introduction

The R package `trio` contains functions for performing genotypic transmission disequilibrium tests (gTDTs) and corresponding score tests to test 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 matched pseudo-controls, i.e. the combinations of alleles that were not transmitted from the parents to their offspring, but were also possible given the parents' genotypes. Additionally, `trio` also comprises a function for applying the allelic TDT (Spielman *et al.*, 1993) to genotype data.

Moreover, `trio` provides functionalities relevant for the analysis of case-parent trio data with *trio logic regression* (Li *et al.*, 2010). These are, on the one hand, 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 on the other hand, functions for applying trio logic regression and a bagging version of trio logic regression to these objects.

Finally, `trio` provides functions for reading in and manipulating the case-parent trio data, estimating LD as well as LD-blocks, and simulating case-parent trio 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 allelic and genotypic TDTs as well as the score tests. While Section 3 contains examples for the application of the gTDT functions to individual SNPs, two-way SNP interactions, and gene-environment interactions, Section 4 briefly shows how the allelic TDT and the score tests related to the genotypic TDTs can be used to test these factors.

Section 5 is devoted to the steps relevant for data processing necessary to generate a matrix suitable as input for trio logic regression, starting from a linkage or genotype file, possibly containing 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.

In Section 6, it is shown how trio logic regression and trioFS (trio Feature Selection; Schwender *et al.*, 2011a), in which bagging with base learner trio logic regression is used to stabilize the search for relevant SNP interactions, can be applied to the data generated as described in Section 5.

For the estimation of the haplotype structure that might be used in the functions described in Section 5, the R package `trio` also contains functions for computing and plotting the

pairwise LD values and for detecting LD blocks. In Section 7, 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 8 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

### 2.1 FamilyExperiment

Here we propose the `FamilyExperiment` class as the container class for experiments involving parent-offspring trios. It is an extension of the `SummarizedExperiment` class defined in the Bioconductor package `GenomicRanges`. Data of this type are typically stored in a ped file, or vcf file. In the case of the latter format one may use tools such as `vcftools` to convert a .vcf file to a .ped file. We show in the following section how to create a `FamilyExperiment` object from a .ped file.

#### 2.1.1 .ped File

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)
> 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

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 columns 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 FamilyExperiment object through matrix coercion.

```
> (fe <- FamilyExperiment(se, pedigree = ped ))

class: FamilyExperiment
dim: 8951 960
exptData():
assays(1): geno
rownames(8951): chr8:129296000 chr8:129296113 ... chr8:130354703
chr8:130354790
rowData metadata column names():
colnames(960): H_ME-DS10776_2-DS10776_2 H_ME-DS10776_3-DS10776_3 ...
H_ME-DS11313_3-DS11313_3 H_ME-DS11313_1-DS11313_1
colData names(1): id
pedigree(4139): famid id fid mid sex dx
complete trios(320):

> geno.mat <- as(fe, "matrix")
> geno.mat[1:5,1:8]
```

```

      [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8]
[1,]    0    0    0    1    0    1    1    1
[2,]    0    0    0    0    0    0    0    0
[3,]    0    0    0    0    0    0    0    0
[4,]    0    0    0    0    0    0    0    1
[5,]    1    1    0    0    0    0    0    0

> 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).

```

> 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 with Genotypic TDTs

A single SNP or two-way interaction can be tested with a gTDT by employing the functions `tdt` and `tdtGxG`. 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

```
> 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

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

Likelihood Ratio Test for Epistatic Interactions Based on Genotypic TDTs

```
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) and Cordell *et al.* (2004). 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 coding variables for each of the two SNPs, and the other additionally containing the four possible interactions of these 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.

Besides this likelihood ratio test (which is the default for the argument `test`), `tdtGxG` also provides the possibility to perform a likelihood ratio test comparing a conditional logistic regression model containing one parameter for each SNP and one parameter for the interaction of these two SNPs with a model only consisting of the two parameters for the main effects of the SNPs (`test = "lrt"`), where the genetic mode of inheritance assumed for the SNPs can be specified by the argument `mode` (by default, an additive mode is assumed). Furthermore, it is also possible to perform a Wald test for the interaction term either by considering a conditional logistic regression model either composed of one parameter for each SNP and one parameter for the interaction (`test = "full"`) or consisting of just one parameter for the interaction (`test = "screen"`).

Thus, if the most simplest of these tests should be performed, then this could be done by

```
> tdtGxG(mat.test[,1], mat.test[,2], test="screen")
```

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	0.030
SNP2	3.1569	0.9772	0.04811	3.15688	0.157
SNP3	2.7150	1.0209	2.71503	0.76851	0.213
SNP4	0.6990	0.3994	0.69897	0.01234	0.691
SNP5	0.2156	0.1950	0.07337	0.21555	0.885

All two-way interactions comprised a matrix in genotype format can be tested using the function `colGxG`. 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. There are several other reasons why this might happen. One of these reasons is that either the minor allele frequencies of both SNPs are very small or that the number of trios influencing the parameter estimation becomes very small when considering the two SNPs in combination.

Therefore, `colGxG` 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 <- colGxG(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

ORs for Exposed Cases:

	OR	Lower	Upper
SNP2	1.0769	0.6315	1.837
SNP1	0.7778	0.4397	1.376
SNP6	1.2667	0.6437	2.493
SNP4	1.0000	0.5538	1.806
SNP5	1.2222	0.6556	2.279

2 df Likelihood Ratio Test, 2 df Wald Test, 1 df Likelihood Ratio Test:

	2df Stat	2df p-Value	Wald Stat	Wald p-value	1df Stat	1df p-Value
SNP2	3.1061	0.2116	3.0096	0.2221	2.1242	0.1450
SNP1	1.1002	0.5769	1.0930	0.5790	1.0577	0.3037
SNP6	4.4641	0.1073	4.2735	0.1180	0.6797	0.4097
SNP4	0.7848	0.6754	0.7781	0.6777	0.3845	0.5352
SNP5	0.4007	0.8185	0.3987	0.8193	0.2055	0.6503

In this situation, a conditional logistic regression model  $\beta_G G + \beta_{G \times E}(G \times E)$  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`.

For both  $\beta_G$  and  $\beta_{G \times E}$ , the same as statistics as, for example, in `colTDT`, are computed. Additionally, the odds ratios and their confidence intervals for the exposed trios are determined (note that the odds ratios for the unexposed trios are given by  $\exp(\beta_G)$ ), and a 2 degree of freedom Wald test for testing both  $\beta_G$  and  $\beta_{G \times E}$  simultaneously as well as two likelihood ratio tests are performed, where the 2 df likelihood ratio test compares the likelihood of the full model (containing both  $\beta_G$  and  $\beta_{G \times E}$ ) with the likelihood of a null model containing no variable, and the 1 df likelihood ratio test compares the likelihoods of the full model and a model only consisting of the SNP. The computation of all these statistics can be avoided by setting `addGandE = FALSE` (for the odds ratio of the exposed cases), `add2df = FALSE` (for the 2 df Wald test) and `whichLRT = "none"` for the two likelihood ratio tests.

If these statistics should be determined, but only the results for the genotypic TDT for testing the gene-environment interaction should be printed, then this can be done 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

A convenient way to generate a data frame containing all the statistics determined by `colGxE` is to use the function `getGxEstats`. This function can be employed to obtain these results for all considered SNPs, and it can also be used to get these statistics only

for the top SNPs. If, for example, the results of the three SNPs showing the smallest p-values for the 2 df likelihood ratio test are of interest, then the data frame containing these SNPs can be generated by

```
> dat.top3 <- getGxEstats(gxe.out, top=3, sortBy="lrt2df")
> dat.top3
```

	Coef GxE	OR GxE	Lower GxE	Upper GxE	SE GxE	Stat GxE	pval GxE
SNP6	-0.3877655	0.6785714	0.2696658	1.707518	0.4708282	0.6782869	0.4101772
SNP2	0.5849336	1.7948718	0.8134061	3.960586	0.4038127	2.0982302	0.1474697
SNP1	-0.4256678	0.6533333	0.2896241	1.473787	0.4150604	1.0517658	0.3051008

	Trios0	Trios1	Coef G	OR G	Lower G	Upper G	SE G	Stat G
SNP6	34	29	0.6241543	1.866667	0.9970286	3.494829	0.3199702	3.8050887
SNP2	37	36	-0.5108256	0.600000	0.3344814	1.076293	0.2981424	2.9356067
SNP1	32	40	0.1743534	1.190476	0.6664394	2.126575	0.2960051	0.3469463

	pval G	OR G&E	Lower G&E	Upper G&E	Stat LRT	2df	pval LRT	2df
SNP6	0.05109707	1.2666667	0.6436665	2.492664	4.464086		0.1073090	
SNP2	0.08664610	1.0769231	0.6314775	1.836587	3.106149		0.2115964	
SNP1	0.55584687	0.7777778	0.4397363	1.375684	1.100231		0.5768831	

	Stat Wald	2df	pval Wald	2df	Stat LRT	1df	pval LRT	1df
SNP6	4.273492		0.1180383		0.6797377		0.4096771	
SNP2	3.009647		0.2220565		2.1241807		0.1449903	
SNP1	1.093011		0.5789694		1.0576748		0.3037461	

## 4 Score Tests and the Allelic TDT

Besides functions for performing genotypic TDTs, trio also provides functions for applying an allelic TDT and score tests related to the genotypic TDTs to matrices in genotype format. For example, the case-parent trio data in `mat.test` can be analyzed with an allelic TDT by

```
> a.out <- allelicTDT(mat.test)
> a.out
```

Allelic TDT

Top 5 SNPs:

	Statistic	p-value
6	3.7532	0.05271
3	1.0253	0.31126
2	0.9804	0.32210

```

4    0.4000 0.52709
5    0.1951 0.65869

```

By default, McNemar's test statistic

$$a = \frac{(b - c)^2}{b + c}$$

is used as test statistic in `allelicTDT`, where  $b$  and  $c$  are the off-diagonal elements of the 2x2-table summarizing the transmitted and untransmitted alleles from heterozygous parents, i.e.  $b$  is the number of heterozygous parents that transmitted the minor allele to their respective children, and  $c$  the number of heterozygous parents that transmitted the major allele. Alternatively, a version of McNemar's test statistic corrected for continuity, namely

$$a_{\text{Cor}} = \frac{(|b - c| - 1)^2}{b + c}$$

can be used by calling

```

> a.out2 <- allelicTDT(mat.test, correct=TRUE)
> a.out2

```

```

Allelic TDT

```

```

Top 5 SNPs:
Statistic p-value
6    3.3247 0.06825
3    0.8101 0.36808
2    0.7941 0.37286
4    0.2778 0.59816
5    0.1098 0.74042

```

If a score test instead of a Wald test, i.e. a genotypic TDT, should be considered in the analysis of genotype data, then `scoreTDT`, `scoreMaxStat`, `scoreGxG`, and `scoreGxE` can be used instead of and in the same way as `colTDT`, `colTDTmaxStat`, `colGxG`, and `colGxE`, respectively. For example, the SNPs in `mat.test` can be tested individually under the assumption of an additive mode of inheritance by

```

> s.out <- scoreTDT(mat.test)
> s.out

```

#### Score Test for Individual SNPs

Model Type: Additive

Top 5 SNPs:

	Score	Statistic	p-value
SNP6	8.5	3.7532	0.05271
SNP3	-4.5	1.0253	0.31126
SNP2	-5.0	0.9804	0.32210
SNP4	-3.0	0.4000	0.52709
SNP5	2.0	0.1951	0.65869

Score tests, however, only provide scores and p-values, but do not allow the computation of odds ratios, relative risks, and confidence intervals. Usually, score tests have the advantage that their test statistic is much faster to compute than the statistic of a Wald test. However, when considering SNPs or interactions between SNPs and binary environmental factors, both the score tests and the genotypic TDTs have about the same computation time, as in these situations, there also exist analytic solutions for the genotypic TDT statistics (see Schwender *et al.*, 2011b).

## 5 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 5.1). If no Mendelian inconsistencies are detected, this function creates an object that is passed to the function `trio.prepare`. 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 7). Given the lengths of the LD blocks, the haplotype frequencies can be estimated, using the function `haplo.em` in the `haplo.stat` package.

## 5.1 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

```
> trio.tmp <- trio.check(dat=trio.ped1)
> #str(trio.tmp, max=1)
> trio.tmp$trio[1:6,]
```

	famid	pid	snp1	snp2	snp3	snp4	snp5	snp6	snp7	snp8	snp9	snp10
1	10001	10001_1	0	0	1	1	1	1	0	0	0	0
2	10001	10001_2	0	2	0	0	2	0	0	0	0	0
3	10001	10001_3	0	1	1	1	1	1	0	0	0	0
4	10002	10002_1	0	0	2	2	0	2	0	0	0	1
5	10002	10002_2	0	2	0	0	2	0	0	0	0	0
6	10002	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 7).

The function `trio.prepare`, 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.prepare` 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. To make the matrix generated by `trio.prepare` reproducible, the function `set.seed` is used to set the random number generator in a reproducible state.

```
> set.seed(123456)
> trio.bin <- trio.prepare(trio.dat=trio.tmp, blocks=c(1,4,2,3))
> #str(trio.bin, max=1)
>
> 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	1	0	1	0	1	0
[4,]	0	0	0	1	0	0	0	0	0	1	1
[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	0
[2,]	0	0	0	0	0	0	0	0	0	0	0
[3,]	0	0	0	0	0	0	0	0	0	0	0
[4,]	1	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	0	0
[7,]	1	0	0	0	0	0	0	0	1	0	0
[8,]	1	0	0	0	0	0	0	0	1	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.prepare`.

```
> #str(trio.gen1)
> 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=FALSE)
> set.seed(123456)
> trio.bin2 <- trio.prepare(trio.dat=trio.tmp, blocks=c(1,4,2,3))
> trio.bin2$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	1	0	1	0	1	0
[4,]	0	0	0	1	0	0	0	0	0	1	1
[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	0
[2,]	0	0	0	0	0	0	0	0	0	0	0
[3,]	0	0	0	0	0	0	0	0	0	0	0
[4,]	1	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	0	0
[7,]	1	0	0	0	0	0	0	0	0	1	0
[8,]	1	0	0	0	0	0	0	0	0	1	0

## 5.2 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:

```
> #str(trio.ped2)
>
> 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.prepare` 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.

```

> set.seed(123456)
> trio.bin3 <- trio.prepare(trio.dat=trio.tmp, blocks=c(1,4,2,3))
> trio.bin3$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	0	0	1	0	1	0	1	0
[4,]	0	0	0	0	0	0	0	0	0	1	1
[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,]	1	0	0	0	0	0	0	0	0	0
[3,]	0	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	0	0
[8,]	1	0	0	0	0	0	0	0	1	0

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

```

> #str(trio.gen2)
> trio.tmp <- trio.check(dat=trio.gen2, is.linkage=FALSE)
> set.seed(123456)
> trio.bin4 <- trio.prepare(trio.dat=trio.tmp, blocks=c(1,4,2,3))
> trio.bin4$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,]	0	0	0	0	0	0	0	0	0	0
[4,]	1	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	0	0
[7,]	0	0	0	0	0	0	0	0	1	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.prepare` 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.

```
> trio.tmp <- trio.check(dat=trio.gen2, is.linkage=FALSE)
> set.seed(123456)
> trio.imp <- trio.prepare(trio.dat=trio.tmp, blocks=c(1,4,2,3), logic=FALSE)
> #str(trio.imp, max=1)
> 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

```
> 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

```
> 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	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:

```
> trio.tmp <- trio.check(dat=trio.ped2)
> set.seed(123456)
> trio.imp2 <- trio.prepare(trio.dat=trio.tmp, blocks=c(1,4,2,3), logic=FALSE)
> trio.imp2$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	1	0	0	2	0	0	0	0	0
6	10002	3	0	1	1	1	1	1	0	0	0	0

### 5.3 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.

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

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

```
> #str(trio.tmp, max=1)
```

```
> 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 these 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)
> 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.

```
> 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

## 5.4 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.prepare` 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:

```
> #str(freq.hap)
> 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.

```
> trio.tmp <- trio.check(dat=trio.gen2, is.linkage=FALSE)
> set.seed(123456)
> trio.imp3 <- trio.prepare(trio.dat=trio.tmp, freq=freq.hap, logic=FALSE)
> #str(trio.imp3, max=1)
> 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

```

```
> trio.imp3$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

```

## 6 Trio Logic Regression

After having prepared a matrix suitable for a trio logic regression analysis, the function `trioLR` can be used to perform this analysis.

To ensure that the following examples are fast to run, we here only use 1000 iterations, i.e. the minimum number of iterations allowed, in the stochastic search algorithms, simulated annealing and MCMC, employed in trio logic regression. **In a real trio logic regression analysis, at least a few hundred thousands of iterations should be used, where the number of iterations in particular depends on the number of variables considered in the analysis.**

The parameter `iter` for setting the number of iterations along with several other control parameters for the two stochastic search algorithms and the logic tree, i.e. the logic expression, grown in a trio logic regression can be specified by employing the argument `control` of the function `trioLR`, which in turn should be specified via the function `lrControl`. Here, we set

```
> my.control <- lrControl(start=1, end=-3, iter=1000, output=-4)
```

where `start` and `end` are the starting and end temperature on log10-scale in simulated annealing, where the temperature governs how likely it is that the model proposed in an iteration of trio logic regression is accepted although it actually performs worse than the model accepted in the previous iteration (at the beginning, this acceptance probability is comparatively high so that many models are visited/accepted, whereas in the last iterations, this probability is very low so that virtually only models are accepted if they are better than the current trio logic regression model). Again, it is very hard to give a good recommendation how to choose `start` and `end` (if they are not specified, the algorithm tries to find good choices for these two parameters, which, however, might work suboptimal), as their optimal specification highly depends on the data at hand. In our experience, `start = 1` and `end = -3` are often reasonable choices for a trio logic regression, but for a particular case-parent trio data set other choices might work better. The help page for the function `logreg.anneal.control` in the R package `LogicReg` gives a comprehensive introduction on how the control parameters of simulated annealing can be chosen.

Finally, we have set `output = -4`, so that the models visited during the search for the best trio logic regression model with an MCMC algorithm are not stored in file, but all statistics for individual SNPs, pairs of SNPs, and triplets of SNPs are computed.

Employing this specification of these four parameters and the defaults for the other control parameters, `trioLR` can be directly applied to the output of `trio.prepare`, i.e. for example `trio.bin` (see Section 5.2), by

```
> lr.out <- trioLR(trio.bin, control=my.control, rand=9876543)
> lr.out
```

```
Trio Logic Regression
```

```
Search Algorithm: Simulated Annealing
```

```
A single model has been fitted:
-2.54 * ((snp6.D | snp2.R) & !snp4.D)
Score: 0.328
```

where we specify the argument `rand` to set the random number generator in a reproducible state. By default, the logic expression found by trio logic regression is printed as it has been found. Alternatively, it can also be printed in disjunctive normal form

```
> print(lr.out, asDNF=TRUE)

      Trio Logic Regression

Search Algorithm: Simulated Annealing

A single model has been fitted:
-2.54 * ((snp2.R & !snp4.D) | (!snp4.D & snp6.D))
Score: 0.328
```

This has the advantage that the interactions – in particular, in a statistical sense – contained in the logic expression are directly given by the AND-combinations (&) in this disjunctive normal form. In the example, this advantage admittedly does not really exist, but when a much larger and more complex logic tree is grown, the logic expression represented by this logic tree can be very hard to interpret.

It is also possible to construct the matrix `x` containing the values of the cases and the matched pseudo-controls and the vector `y` comprising the class labels of the (artificial) subjects without using `trio.prepare`. In this situation, each column of `x` must represent one of the logic/binary variable, coded by zeros and ones, and each row must correspond to a case or a pseudo-control, where each block of four consecutive rows has to consist of the data for a case and the three matched pseudo-controls (in this order). In `y`, each case must be coded by a 3 and each pseudo-control by a 0 so that `y` is given by

```
> n.trios <- 100
> y <- rep(c(3, 0, 0, 0), n.trios)
```

where `n.trios` is the number of case-parent trios considered in a study. This number is here set to 100, as our example data set `trio.bin` consists of 100 trios. To avoid the construction of the matrix `x`, we extract this matrix for this example from `trio.bin`.

```
> x <- trio.bin$bin[,-1]
```

The same trio regression analysis as above can then be performed by

```
> lr.out2 <- trioLR(x, y, control=my.control, rand=9876543)
> lr.out2
```

Trio Logic Regression

Search Algorithm: Simulated Annealing

A single model has been fitted:

-2.54 \* ((snp6.D | snp2.R) & !snp4.D)

Score: 0.328

The trio logic regression model resulting from such an application can then be tested by a null-model permutation procedure, which checks whether there is signal in the data, or by a conditional permutation test for model selection. Both can be performed using the function `trio.permTest` in which the argument `conditional` specifies which of these permutation tests are done. Thus, the null-model permutation test with `n.perm = 20` permutations can be applied to the case-parent trio data in `trio.bin` by

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

while the conditional permutation test can be performed by

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

By default, (trio) logic regression uses simulated annealing as search algorithm, and `trioLR` fits one trio logic regression model comprising one logic tree with a maximum of five leaves, i.e. five binary variables coded by zeros and ones. Thus, the argument `nleaves` is by default set to `nleaves = 5`, whereas the number of logic trees cannot be changed in a trio logic regression.

Alternatively, several trio logic regression models with different maximum numbers of leaves/variables can be fitted by setting `nleaves` to a vector of length 2, where the first element specifies the lowest maximum number, and the second element the largest

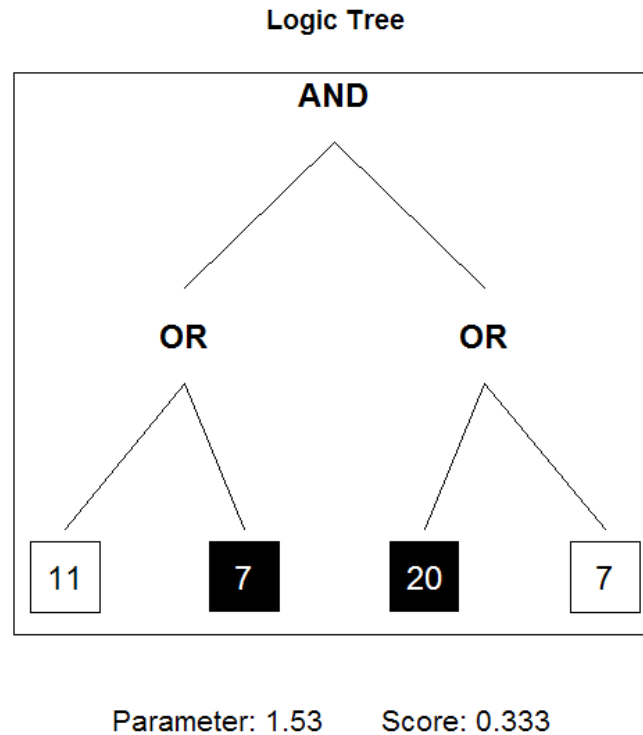


Figure 1: Logic tree built in a trio logic regression analysis of case-parent trio data in which a maximum of five leaves was allowed.

maximum number. If thus, for example, trio logic regression models should be fitted in which the maximum number of leaves varies between 3 and 5, then this can be done by

```
> lr.out3 <- trioLR(trio.bin, nleaves=c(3,5), control=my.control, rand=9876543)
```

```
The number of trees in these models is 1
```

```
The model size is 3
```

```
The best model with 1 trees of size 3 has a score of 0.3278
```

```
The model size is 4
```

```
The best model with 1 trees of size 4 has a score of 0.3269
```

```
The model size is 5
```

```
The best model with 1 trees of size 5 has a score of 0.3325
```

```
> lr.out3
```

```
Trio Logic Regression
```

```
Search Algorithm: Simulated Annealing
```

```
3 models have been fitted:
```

```
Model with a maximum of 3 leaves:
```

```
2.54 * ((!snp2.R & !snp6.D) | snp3.D)
```

```
Score: 0.328
```

```
Model with a maximum of 4 leaves:
```

```
-2.08 * ((!snp8.D | !snp7.D) | snp4.R)
```

```
Score: 0.327
```

```
Model with a maximum of 5 leaves:
```

```
1.53 * ((snp6.D | !snp4.D) & (!snp10.R | snp4.D))
```

```
Score: 0.333
```

The resulting logic trees cannot only be printed, but also plotted. If, for example, the logic tree in the third trio logic regression model (i.e. the model with a maximum of five leaves) should be plotted, then this can be done by

```
plot(lr.out3, whichTree=3)
```

(see Figure 1), where the argument `whichTree` needs only to be specified if several models have been fitted.

Alternatively to simulated annealing, a greedy search can be employed in trio logic regression by changing the argument `search` to "greedy".

```
> lr.out4 <- trioLR(trio.bin, search="greedy", rand=9876543)
```

```
Model 0 has a score of 0.3466
```

```
Model 1 has a score of 0.3429
```

```
Model 2 has a score of 0.3359
```

```
Model 3 has a score of 0.3295
```

```
Model 4 has a score of 0.3253
```

```
Model 5 has a score of 0.3211
```

```
> lr.out4
```

```
Trio Logic Regression
```

```
Search Algorithm: Greedy
```

6 models have been fitted:

Model with 0 leaves:  
Model contains no variables.  
Score: 0.347

Model with a maximum of 1 leaf:  
0.762 \* snp9.D  
Score: 0.343

Model with a maximum of 2 leaves:  
1.43 \* (snp9.D & !snp3.R)  
Score: 0.336

Model with a maximum of 3 leaves:  
1.98 \* ((snp9.D & snp7.D) & !snp3.R)  
Score: 0.33

Model with a maximum of 4 leaves:  
2.1 \* ((snp9.D & (snp7.D | !snp8.D)) & !snp3.R)  
Score: 0.325

Model with a maximum of 5 leaves:  
2.43 \* ((snp9.D & (snp7.D | !snp8.D)) & (!snp3.R & !snp6.R))  
Score: 0.321

While trio logic regression based on simulated annealing or a greedy search tries to identify the logic expression, and thus, the trio logic regression model, that provides the best prediction for the disease risk, the main goal of trio MC logic regression, i.e. trio logic regression based on MCMC is the specification of the individual relevance of SNPs and the joint importance of pairs and triplets of SNPs for disease risk by counting how often the individual SNPs occur in the models visited (and accepted) during the MCMC search and how often pairs and triplets of SNPs occur jointly in these models (cf. Kooperberg and Ruczinski, 2005). This trio MC logic regression can be performed by

```
> lr.out5 <- trioLR(trio.bin, search="mcmc", control=my.control, rand=9876543)
> lr.out5
```

Trio Logic Regression

Search Algorithm: MCMC

Visited models are not stored.



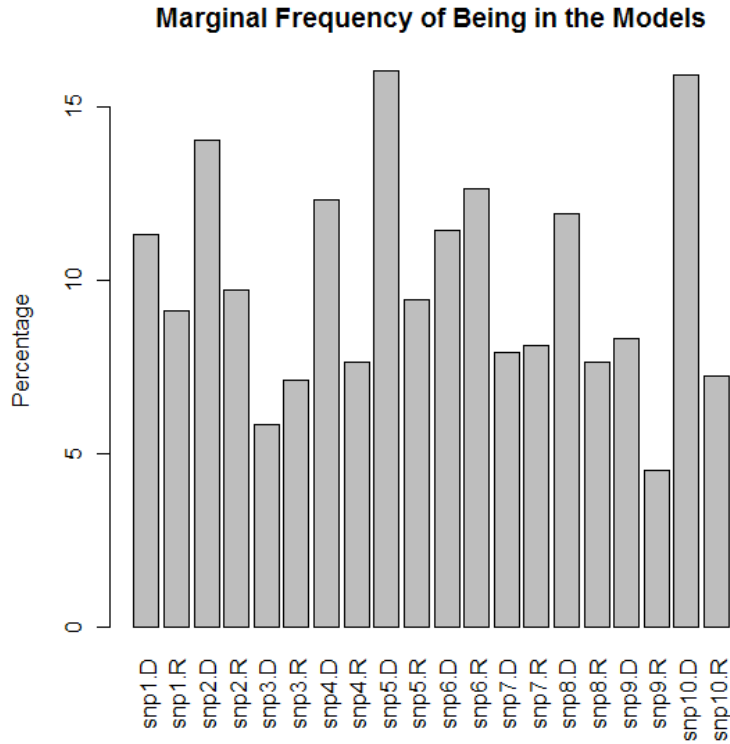


Figure 2: Visualization of the results of a trio MC logic regression analysis for the individual SNPs.

The results of this analysis can be visualized by plotting for each SNP the percentages of visited models in which this SNP occurs (`freqType = 1`; the default), and for each pair of SNPs in how many of the visited models this pair cocurs jointly (`freqType = 2`) and the observed-to-expected ratio of being jointly in the models (`freqType = 3`). For example, in Figure 2,

```
plot(lr.out5, freqType=1, useNames=TRUE)
```

the frequencies for the individual SNPs are displayed.

Another way to quantify the importance of SNPs and SNP interactions for the prediction of the disease risk is to apply trio logic regression to several subsets of the case-parent trio data and then employ the resulting trio logic regression models to compute importance

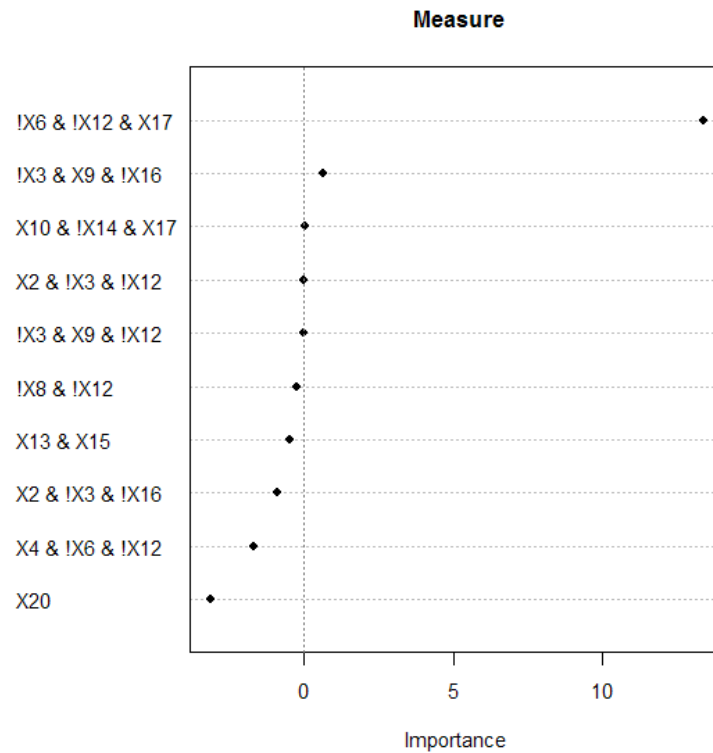


Figure 3: Values of the importance measure of trioFS for the five most important identified SNP interactions.

measures for the interactions found. This procedure called trioFS (trio Feature Selection) is implemented in the function `trioFS` that can be used in a similar way as `trioLR`. For example, trioFS can be applied to the case-parent trio data by

```
> fs.out <- trioFS(trio.bin, B=5, control=my.control, rand=9876543)
> fs.out
```

Identification of Interactions Using Trio Logic Regression

```
Number of Iterations: 5
Sampling Method:      Bagging
Number of Trees:      1
Max. Number of Leaves: 5
```

The 5 Most Important Interactions:

	Importance	Proportion	Expression
1	13.369	0.20	!snp3.R & !snp6.R & snp9.D
2	0.621	0.20	!snp2.D & snp5.D & !snp8.R
3	0.028	0.20	snp5.R & !snp7.R & snp9.D

4	0.000	0.20 snp1.R & !snp2.D & !snp6.R
5	0.000	0.20 !snp2.D & snp5.D & !snp6.R

where we here consider only  $B = 5$  subsets of the data, while in a real analysis, at least  $B = 20$  subsets should be considered. By default, simulated annealing is used in this search. Alternatively, it is also possible to do a greedy search by setting the argument `fast` of `trioFS` to `TRUE`.

The importances of the identified SNP interactions cannot only be printed, but also be plotted.

```
plot(fs.out)
```

(see Figure 3).

## 7 Detection of LD Blocks

For the estimation of the haplotype structure that might be used in the R function `trio.prepare`, 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

```
> 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

```
> 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 4). This figure shows the  $r^2$ -values. The  $D'$  values can be plotted by

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

(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
```

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
```

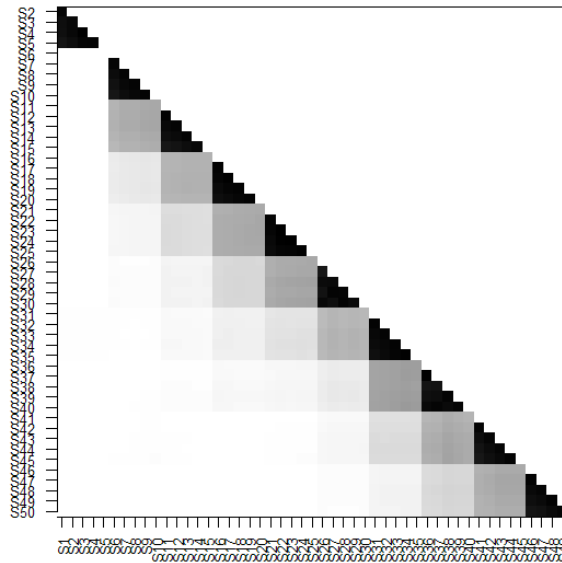


Figure 4: Pairwise  $r^2$  values for the SNPs from LDdata.

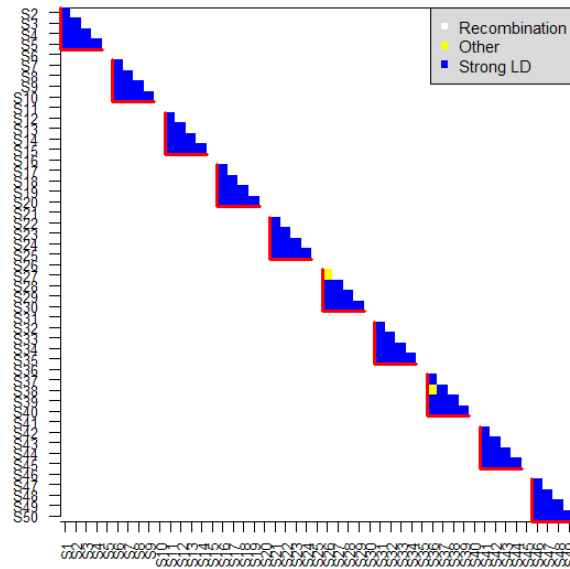


Figure 5: LD blocks as found by the modified algorithm of Gabriel *et al.* (2002). The borders of the LD blocks are marked by red lines. The color for the LD between each pair of SNPs is defined by the three categories used by Gabriel *et al.* (2002) to define the LD blocks.

The blocks can also be plotted by

```
> plot(blocks)
```

(see Figure 5). 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 6).

As mentioned in Section 5, the haplotype structure required by `trio.prepare` can be obtained by

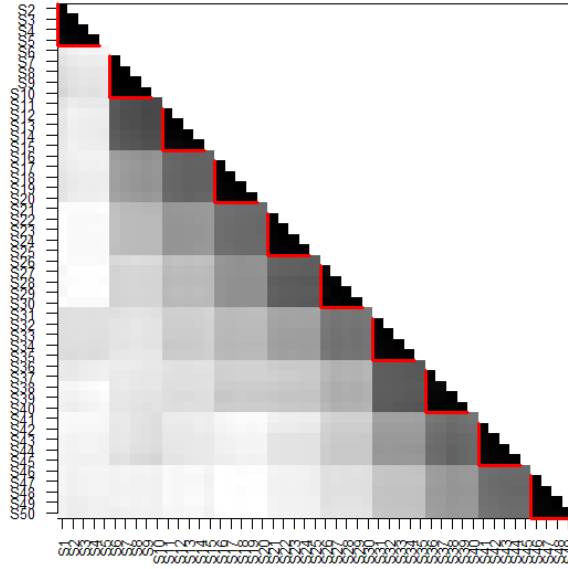


Figure 6: LD blocks as found by the modified algorithm of Gabriel *et al.* (2002). The borders of the LD blocks are marked by red lines. The darker the field for each pair of SNPs, the larger is the  $D'$  value for the corresponding SNP pair.

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

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

## 8 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.

## 8.1 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.

```
> #str(simuBkMap)
> 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)
> sim[[1]][1:6, 1:12]
```

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



## 8.2 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.

```
> trio.tmp <- trio.check(dat=trio.gen1, is.linkage=FALSE)
> trio.impu <- trio.prepare(trio.dat=trio.tmp, blocks=c(1,4,2,3), logic=TRUE)
> #str(trio.impu, max=2)
> trio.impu$freq[1:7,]
```

	key	hap	freq
1	ch-1	1	9.425000e-01
2	ch-1	2	5.750000e-02
3	ch-h2	1111	3.250000e-01
4	ch-h2	1112	2.225000e-01
5	ch-h2	1121	7.500000e-03
6	ch-h2	1221	3.350000e-01
7	ch-h2	1222	3.665409e-09

```
> sim <- trio.sim(freq=trio.impu$freq, interaction="1R and 5R", prev=.001, OR=2,
+ n=20, rep=1)
> #str(sim)
> sim[[1]][1:6, ]
```

	famid	pid	snp1	snp2	snp3	snp4	snp5
[1,]	1	1	2	2	1	1	2
[2,]	1	2	1	2	1	1	2
[3,]	1	3	2	2	0	0	2
[4,]	2	1	2	2	0	0	2
[5,]	2	2	2	2	1	1	1
[6,]	2	3	2	2	0	0	2

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.

```
> sim <- trio.sim(freq=freq.hap, interaction="1R or 4D", prev=.001, OR=2,
+ n=10, rep=2)
> #str(sim)
> sim[[1]][1:6,]
```

	famid	pid	snp1	snp2	snp3	snp4	snp5
[1,]	1	1	2	2	1	1	1

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

### 8.3 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.

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

	famid	pid	snp1	snp2	snp3	snp4	snp5
[1,]	1	1	2	1	1	1	1
[2,]	1	2	1	1	1	1	1
[3,]	1	3	2	1	1	1	1
[4,]	2	1	1	2	0	0	2

```

[5,]      2      2      2      2      2      2      1
[6,]      2      3      1      2      1      1      2

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

      famid pid snp1 snp2 snp3 snp4 snp5
[1,]      1   1   2   2   2   2   1
[2,]      1   2   2   2   1   1   2
[3,]      1   3   2   2   1   1   1
[4,]      2   1   2   2   1   1   1
[5,]      2   2   1   2   1   1   1
[6,]      2   3   2   2   0   0   2

```

## Acknowledgments

Financial support was provided by DFG grants SCHW 1508/1-1, SCHW 1508/2-1, and SCHW 1508/3-1, as well as NIH grants R01 DK061662 and R01 HL090577.

## References

- Cordell, H.J. (2002). Epistasis: What it Means, what it Doesn't Mean, and Statistical Methods to Detect it in Humans. *Human Molecular Genetics*, **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. *Genetic Epidemiology*, **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.

- Kooperberg, C. and Ruczinski, I. (2005). Identifying Interacting SNPs Using Monte Carlo Logic Regression. *Genetic Epidemiology*, **28**, 157–170.
- 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. *Genetic Epidemiology*, **34**, 396–406.
- Schaid, D.J. (1996). General Score Tests for Associations of Genetic Markers with Disease Using Cases and Their Parents. *Genetic Epidemiology*, **13**, 423–449.
- Schwender, H., Bowers, K., Fallin, M.D., and Ruczinski, I. (2011). Importance Measure for Epistatic Interactions in Case-Parent Trios. *Annals of Human Genetics*, **75**, 122–132.
- Schwender, H., Taub, M.A., Beaty, T.H., Marazita, M.L., and Ruczinski, I. (2011). Rapid Testing of SNPs and Gene-Environment Interactions in Case-Parent Trio Data Based on Exact Analytic Parameter Estimation. *Biometrics*. DOI: 10.1111/j.1541-0420.2011.01713.x.
- Spielman, R.S., McGinnis, R.E., and Ewens, W.J. (1993). Transmission Test for Linkage Disequilibrium: The Insulin Gene Region and Insulin-Dependent Diabetes Mellitus (IDDM). *American Journal of Human Genetics*, **52**, 506–516.