

# Family Based Association Tests Using the **fbat** package

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# 1 Introduction

The R package `fbat` can be used to test the following null hypotheses for each marker based on family pedigrees:

- $H_{01}$  : the marker has no association and no linkage with the trait;
- $H_{02}$  : the marker has no association with the trait in the presence of linkage.

We assume that

- the families are **nuclear** families
- there are no missing genotypes and phenotypes for children
- markers are bi-allelic.

A more general software FBAT is available as a stand-alone executable with documentation and example files from <http://www.biostat.harvard.edu/~fbat/fbat.htm>. While this R package has some important limitations as present, these will be addressed in further versions.

# 2 Pedigree data file format

All fields are separated by whitespace (e.g. one or more spaces).

**First line** : names of all markers in the sequence of the genotype data. For example, `marker1, marker2, . . . , markerm`.

**Remaining lines**: The remaining lines contain only non-negative integers and have the same format:

family	pid	father	mother	sex	affection	marker <sub>1.1</sub>	marker <sub>1.2</sub>	...	marker <sub>m.1</sub>	marker <sub>m.2</sub>
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where

**family**: family id

**pid**: patient id

**father**: father id.

Use 0 (zero) for founders or marry-ins (parents not specified) in a pedigree. A **founder** in a pedigree is an individual who is not a child of any individuals in the pedigree.

**mother:** mother id.

Use 0 (zero) for founders or marry-ins (parents not specified) in a pedigree.

A **founder** in a pedigree is an individual who is not a child of any individuals in the pedigree.

**sex:** 1 – male; 2 – female;

**affection:** affection status (i.e., trait)

2 – affected; 1 – unaffected; 0 – unknown

**marker<sub>*i,j*</sub>:** allele *j* of marker *i*,  $j = 1, 2$ ;  $i = 1, 2, \dots, m$ .

non-missing Alleles are represented by positive integers. Missing alleles are represented by zero (0).

### 3 Data quality control

The R package `fbat` also provides some basic QC functions.

The function `missGFreq` checks the completeness of genotypes. This function outputs counts of missing genotypes per marker and per subject.

The function `pedHardyWeinberg` checks the assumption of the Hardy-Weinberg equilibrium for markers.

The function `checkMendelian` checks the following possible Mendelian-related errors:

1. father id = subject id;
2. mother id = subject id;
3. could not determine if an individual is a parent or a child in a family;
4. inconsistent parental sex in a family;
5. parental genotypes are not compatible with childrens' genotypes in a family;
6. all childrens' genotypes are missing in a family;
7. inconsistent sib genotypes in a family.

### 4 Examples

To call the functions in the R package `fbat`, we first need to load it into R:

To read the pedigree file `CAMP.ped` into R, we use the function `readGenes` in the R package `GeneticsBase`:

```
gSet<-readGenes(gfile="CAMP.ped", gformat="fbat")
```

The function `readGenes.ped` returns back an object of the R class *geneSet*.

Before we apply family based association tests, it would be good practice to check Hardy-Weinberg equilibrium for each marker based on parental data. We can use the function `pedHardyWeinberg` to do this.

```
> data(CAMP)
```

```
Reading 8 markers and 2011 subjects from `CAMP.ped` ...
generating 'geneSet' object...
```

```
Successfully read the pedigree file `CAMP.ped`.
```

```
Number of Markers: 8
Number of Subjects: 2011
Number of Families: 651
```

```
Reading 12 vars from `CAMPZ.phe` ... Done.
```

```
Number of Phenotype Variables: 12
Number of Observations      : 2011
```

```
> ch <- pedHardyWeinberg(CAMP)
```

```
converting geneSet object to numerical matrix...
```

```
HWE test...
```

	nInfoInd	nGenotype	nHET	nHOM	nAllele	nMissing	chi2	df	p-value
m709	1271	3	6	1265	2	32	0.007	1	0.933
m654	1265	3	544	721	2	38	5.728	1	0.017
m47	1250	3	585	665	2	53	0.000	1	0.985
p46	1251	3	582	669	2	52	1.165	1	0.280
p79	1244	3	572	672	2	59	0.693	1	0.405
p252	1168	3	377	791	2	135	4.715	1	0.030
p491	1268	3	31	1237	2	35	0.194	1	0.659
p523	1269	3	377	892	2	34	1.377	1	0.241

The column `nInfoInd` means the number of informative individuals, i.e. individuals whose genotypes contain no missing alleles for the specified marker; the column `nGenotype` means number of possible genotypes; the column `nHET` means number of heterozygous genotypes; the column `nHOM` means number of homozygous genotypes; the column `nAllele` means number of alleles; the column `nMissing` means number of missing alleles; the column `chi2` means chi square test statistic; the column means `df` means degree of freedom of the chi square test statistic under the null hypothesis that Hardy-Weinberg condition holds; and the column `p-value` means pvalue of the test.

To view the statistics for individual markers, we can use the function `viewHW`. For example,

```
> viewHW(ch, "p79")

number of possible genotypes for marker p79 >>
[1] 3
genotype frequency >>
  p79.1 p79.2 freq
[1,]    1    1 486
[2,]    1    2 572
[3,]    2    2 186
allele frequency >>
  1    2
0.621 0.379
nInfoInd nGenotype    nHET    nHOM  nAllele nMissing    chi2    df
1244.000    3.000  572.000  672.000    2.000   59.000    0.693    1.000
p-value
0.405
```

To check Mendelian-related errors, we can use the function `checkMendelian`. For example,

```
> tmp <- checkMendelian(CAMP, quiet = TRUE)
> cat("For each marker, how many families contains mendelian errors?\n")

For each marker, how many families contains mendelian errors?

> print(tmp$nMerrMarker)

m709 m654  m47  p46  p79 p252 p491 p523
  20 159 160 155 160 140  26 122

> cat("For each family, how many markers contains mendelian errors?\n")

For each family, how many markers contains mendelian errors?

> cat("tmp$nMerrFamily[1:10]>>\n")

tmp$nMerrFamily[1:10]>>

> print(tmp$nMerrFamily[1:10])

family1 family2 family3 family4 family5 family6 family7 family8
      0      4      4      2      2      2      0      4
family9 family10
      4      1
```

```

> cat("For each family, how many times\n")

For each family, how many times

> cat("'father id = subject id' or 'mother id = subejct id'?\n")

'father id = subject id' or 'mother id = subejct id'?

> cat("tmp$nErrFamilySample[1:10]>>\n")

tmp$nErrFamilySample[1:10]>>

> print(tmp$nErrFamilySample[1:10])

family1 family2 family3 family4 family5 family6 family7 family8
      0      0      0      0      0      0      0      0
family9 family10
      0      0

```

To count the number of missing genotypes for a marker or for a subject, we can use the function `missGFreq`. For example,

```

> res <- missGFreq(CAMP, founderOnly = FALSE, quiet = TRUE)
> cat("The number of missing genotypes for markers>>")

The number of missing genotypes for markers>>

> print(res$nMissMarkers)

      00 0* *0
m709 55 0 0
m654 60 0 0
m47 89 0 0
p46 68 0 0
p79 90 0 0
p252 188 0 0
p491 57 0 0
p523 53 0 0

> cat("The number of missing genotypes for the first 10 subjects>>")

The number of missing genotypes for the first 10 subjects>>

> print(res$nMissSubjects[1:10, ])

```

```

          00 0* *0
subject1  0  0  0
subject2  0  0  0
subject3  0  0  0
subject4  0  0  0
subject5  0  0  0
subject6  1  0  0
subject7  0  0  0
subject8  0  0  0
subject9  0  0  0
subject10 0  0  0

```

To get the family based association test statistics, we use the function `fbat`:

```
> res <- fbat(CAMP)
```

The usage of the function `fbat` is

```
fbat(geneSetObject, model="a", traitMethod=3, traitOffset=0, quiet=TRUE)
```

The function argument `model` specifies the genotype codings.

By default, we use the additive model (`model="a"`). Other available models include dominant (`model="d"`), recessive (`model="r"`), and genotype (`model="g"`) models.

The function argument `traitMethod` indicates the trait coding method. If `traitMethod` is equal to 1, then the trait is represented by `trait-offset` where `trait` is the sixth column (i.e., affection status) of the pedigree matrix and the value of `offset` is provided by the argument `traitOffset`. If the argument `traitMethod` takes value other than 1, then the trait is set to be 1 if the sixth column of the pedigree matrix takes value 2 and the trait is set to be 0 if the sixth column of the pedigree matrix takes value 1.

The function `fbat` returns a list. To summarize the values, degrees of freedom, and *p*-values of the test statistics for the markers, we can use the function `summaryPvalue`:

```
> summaryPvalue(res)
```

```

*****
      chisq rank      pvalue
m709  1.285714    1 2.568393e-01
m654  9.570312    1 1.977491e-03
m47   16.106145    1 5.988938e-05
p46   10.029304    1 1.540692e-03
p79   10.267823    1 1.353705e-03
p252  20.000000    1 7.744216e-06
p491  12.448276    1 4.183778e-04
p523  26.097826    1 3.245492e-07
*****

```

To adjust multiple comparisons, we can use the function `p.adjust` in the R package `base` to adjust the  $p$ -values. For example,

```
> pvals <- res$statPvalue[, 3]
> p.adjust.M <- p.adjust.methods
> p.adj <- sapply(p.adjust.M, function(meth) p.adjust(pvals, meth))
> noquote(apply(p.adj, 2, format.pval, digits = 3))
```

	holm	hochberg	hommel	bonferroni	BH	BY	fdr	none
[1,]	0.256839	0.256839	0.256839	1.000000	0.256839	0.698052	0.256839	0.256839
[2,]	0.005415	0.003955	0.003955	0.015820	0.002260	0.006142	0.002260	0.001977
[3,]	0.000359	0.000359	0.000359	0.000479	0.000160	0.000434	0.000160	5.99e-05
[4,]	0.005415	0.003955	0.003081	0.012326	0.002054	0.005583	0.002054	0.001541
[5,]	0.005415	0.003955	0.002966	0.010830	0.002054	0.005583	0.002054	0.001354
[6,]	5.42e-05	5.42e-05	5.42e-05	6.20e-05	3.10e-05	8.42e-05	3.10e-05	7.74e-06
[7,]	0.002092	0.002092	0.002092	0.003347	0.000837	0.002274	0.000837	0.000418
[8,]	2.60e-06	2.60e-06	2.60e-06	2.60e-06	2.60e-06	7.06e-06	2.60e-06	3.25e-07

To view summary statistics of individual marker, we can use the function `viewstat`. For example,

```
> viewstat(res, "p79")

*****
651 pedigree 2011 persons
360 informative families at marker p79
The alleles of marker p79 >>
[1] 1 2
Score for marker p79 >>
[1] 474 298
Expected score for marker p79 >>
[1] 437.5 334.5
Covariance matrix of the score for marker p79 >>
      [,1] [,2]
[1,] 129.75 -129.75
[2,] -129.75 129.75
Moore-Penrose generalized inverse of covariance matrix
      [,1] [,2]
[1,] 0.001926782 -0.001926782
[2,] -0.001926782 0.001926782
test statistics for marker p79 >>
      chisq      rank      pvalue
10.267822736 1.000000000 0.001353705
*****
```



Note that if the covariance matrix of the S score vector is singular, the Moore-Penrose generalized inverse is used.

Sometimes the user might want to know if a genotype is homozygous or heterozygous. The function `pedFlagHomo` can provide those information. For example,

```
> res.f <- pedFlagHomo(CAMP)

converting geneSet object to numerical matrix...
flag homozygotes and heterozygotes...
dim(flagHomoMat)= 1303 8
length(ped[,2])= 1303
numHomo -- number of homozygous genotypes
numHetero -- number of heterozygous genotypes
numMiss1 -- number of genotypes containing one missing allele
numMiss2 -- number of genotypes containing two missing alleles
counts>>>
      numHomo numHetero numMiss1 numMiss2
m709    1265         6         0         32
m654     721        544         0         38
m47      665        585         0         53
p46      669        582         0         52
p79      672        572         0         59
p252     791        377         0        135
p491    1237         31         0         35
p523     892        377         0         34
```

The function `pedGFreq` gets genotype frequencies and percentages. For example,

```
> res <- pedGFreq(CAMP)

converting geneSet object to numerical matrix...
counting genotype frequencies...
genotype counts>>>
      1/1 1/2 2/2
m709 1265  6  0
m654  537 544 184
m47   175 585 490
p46   209 582 460
p79   486 572 186
p252   70 377 721
p491 1237  31  0
p523  840 377  52
```

The function `pedAFreq` gets allele frequencies and percentages. For example,

```

> res <- pedAFreq(CAMP)

converting geneSet object to numerical matrix...
count allele frequencies...
allele frequencies and percentages>>>
      1    2    1    2
m709 2536    6 0.998 0.002
m654 1618   92 0.640 0.360
m47   935 1565 0.374 0.626
p46  1000 1502 0.400 0.600
p79  1544   94 0.621 0.379
p252  517 1819 0.221 0.779
p491 2505   31 0.988 0.012
p523 2057   48 0.810 0.190

```

The functions `fbat`, `pedHardyWeinberg`, `pedFlagHomo`, `pedGFreq`, and `pedAFreq` have default forms (`fbat.default`, `pedHardyWeinberg.default`, `pedFlagHomo.default`, `pedGFreq.default`, and `pedAFreq.default`) that use a pedigree matrix as input.

## Appendix

### A Notation

For a given marker,

- $Y_{ij}$  — Observed trait of the  $j$ -th offspring in family  $i$ .
- $T_{ij}$  — A function of  $Y_{ij}$ .

$$T_{ij} = T(Y_{ij}).$$

For example

$$T_{ij} = T(Y_{ij}) = Y_{ij} - \mu_{ij},$$

where  $\mu_{ij}$  is an offset.

- $g_{ij}$  — Genotype of the  $j$ -th offspring in family  $i$ ;
- $X_{ij}$  — A function of  $g_{ij}$ .

$$X_{ij} = X(g_{ij}).$$

- $S$  score:

$$S = \sum_{ij} T_{ij} X_{ij} = \sum_{ij} T(Y_{ij}) X(g_{ij}).$$

- test statistic:

$$U = S - E[S|H_0, \mathcal{C}],$$

where  $\mathcal{C}$  is a condition set. When parental genotypes are complete, the condition set  $\mathcal{C} = \mathcal{T} \cup \mathcal{G}$ , where  $\mathcal{T}$  is the observed traits in all family members and  $\mathcal{G}$  is the parental genotypes. When parental genotypes are incomplete, the condition set  $\mathcal{C} = \mathcal{T} \cup \mathcal{G}^* \cup \mathcal{G}_{\text{offspring}}$ ,  $\mathcal{G}^*$  is the partially observed parental genotypes and  $\mathcal{G}_{\text{offspring}}$  is the set of offspring genotypes (i.e., the offspring genotype configuration).

- $V$  – variance or covariance matrix of  $U$  under the null hypothesis  $H_0$ . I.e.,

$$V = \text{Cov}(U|H_0, \mathcal{C}) = \text{Cov}(S|H_0, \mathcal{C}).$$

- For the univariate case,

$$Z = \frac{U}{\sqrt{V}} \Big|_{H_0, \mathcal{C}} \dot{\rightarrow} N(0, 1).$$

- For the multivariate case,

$$\chi^2 = U'V^{-1}U \Big|_{H_0, \mathcal{C}} \dot{\rightarrow} \chi_r^2,$$

where  $r = \text{rank}(V)$ .

## B Genotype coding methods

Denote  $K$  as the number of all possible different alleles for the locus and  $X$  as the vector of genotype coding.

**GEN**  $X$  is a vector with length equal to the number of genotypes that are possible given the parental genotypes in the sample, a maximum of  $K(K+1)/2$  genotypes, and with elements equal to 1 or 0 to indicate which of the possible genotypes is equal to the genotype  $g$ .

**GDOM** codes the  $j$ th element of the vector  $X$  as  $x_j = 1$  if genotype  $g$  has one or two alleles of type  $j$ , otherwise  $x_j = 0$ .  $X$  is a vector of length  $K$ .

**GREC** codes the  $j$ th element of the vector  $X$  as  $x_j = 1$  if genotype  $g$  has two alleles of type  $j$ , otherwise  $x_j = 0$ .  $X$  is a vector of length  $K$ .

**GTDT** scores the number of alleles of a particular type by coding  $x_j$  equal to the number of alleles of type  $j$  in the genotype  $g$  (i.e.,  $x_j = 0, 1$ , or  $2$  if  $g$  has  $0, 1$  or  $2$  alleles of type  $j$ ).  $X$  is a vector of length  $K$ .

## 2-allele case

Example of different marker codings for a marker with  $K = 2$  alleles, see Schaid (1996)

genotype	$X(g)$			
$g$	GEN	GDOM	GREC	GTDT
		$(A, a)$	$(A, a)$	$(A, a)$
$AA$	$(0,0,0)$	$(1,0)$	$(1,0)$	$(2,0)$
$Aa$	$(1,0,0)$	$(1,1)$	$(0,0)$	$(1,1)$
$aa$	$(0,1,0)$	$(0,1)$	$(0,1)$	$(0,2)$

## 3-allele case

Example of different marker codings for a marker with  $K = 3$  alleles, see Schaid (1996) (This table is Table 4 of Horvath et al.'s report for FBAT software)

genotype	$X(g)$			
$g$	GEN	GDOM	GREC	GTDT
		$(A, B, C)$	$(A, B, C)$	$(A, B, C)$
$AA$	$(0,0,0,0,0)$	$(1,0,0)$	$(1,0,0)$	$(2,0,0)$
$AB$	$(1,0,0,0,0)$	$(1,1,0)$	$(0,0,0)$	$(1,1,0)$
$AC$	$(0,1,0,0,0)$	$(1,0,1)$	$(0,0,0)$	$(1,0,1)$
$BB$	$(0,0,1,0,0)$	$(0,1,0)$	$(0,1,0)$	$(0,2,0)$
$BC$	$(0,0,0,1,0)$	$(0,1,1)$	$(0,0,0)$	$(0,1,1)$
$CC$	$(0,0,0,0,1)$	$(0,0,1)$	$(0,0,1)$	$(0,0,2)$

## C Trait coding methods

Denote  $Y_{ij}$  as the trait of the  $j$ -th child of the  $i$ th nuclear family.  $Y_{ij}$  can be dichotomous, measured (i.e., continuous?), time-to-onset (i.e., censored?)

The trait coding methods ( $T_{ij} = T(Y_{ij})$ ) are listed below:

- $T_{ij} = 1$  if the  $j$ th child is affected;  $T_{ij} = 0$  otherwise.
- $T_{ij} = Y_{ij} - \mu_{ij}$ , where  $\mu_{ij}$  is an offset.
- $T_{ij} = Y_{ij} - \mu_{ij}(\mathbf{x}'\boldsymbol{\beta})$ , where  $E(Y_{ij}|\mathbf{x}) = \mu_{ij}(\mathbf{x}'\boldsymbol{\beta})$ , and  $\mathbf{x}$  are design matrix of covariates,  $\boldsymbol{\beta}$  are unknown parameters.