

PedGFLMM Vignette

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1 Overview

This document describes our R package **PedGFLMM** which implements family-based additive generalized linear mixed models (GLMM) and generalized functional linear mixed models (GFLMM) for gene-based association testing of dichotomous traits (Jiang et al, 2020). Section 2 briefly describes the installation of the program. Section 3 describes the data formats. Section 4 explains how to run the program using one example. Section 5 offers explanation of the results and warnings to use the programs. Section 6 provides some suggestions and parameter choices for real data analysis.

The theoretical basis for this program is given in our research papers in Section 7 “References” below; the primary paper describing this work is Jiang et al (2020). Please cite the references if you use our program in any published work. In case of suggestions and questions and/or problems, you can contact us via e-mail (rf740@georgetown.edu).

An HTML version of this vignette can be found at <https://github.com/DanielEWeeks/PedGFLMM>.

2 Installation

The package is written in R language. To install, proceed as follows:

1. Install the `devtools` package by starting up R and issuing this command:

```
install.packages("devtools")
```

2. Load the `devtools` library to make its commands available:

```
library(devtools)
```

3. Install the `PedGFLMM` R package from the github repository via this command:

```
install_github("DanielEWeeks/PedGFLMM/pkg")
```

If you wish to have this vignette installed and accessible within your R help pages, use this command instead (but note that this will be slower):

```
install_github("DanielEWeeks/PedGFLMM/pkg", build_opts = c("--no-resave-data", "--no-manual"),  
  build_vignettes = TRUE)
```

Note that this vignette is available online at <https://github.com/DanielEWeeks/PedGFLMM>.

After the `PedGFLMM` R package has been installed, you can view this vignette by issuing these commands at the R prompt:

```
library(PedGFLMM)  
browseVignettes("PedGFLMM")
```

3 Data Format

The program needs data frame in R to define the pedigree structure (typical format used by LINKAGE and PLINK), genotypes, SNP positions, and covariates.

3.1 The pedigree file

The pedigree file is in the same format as that used by the `pedgene` R package except for a column named ID (Schaid et al. 2013) and has the following columns:

- ID: identify of each individual.
- ped: pedigree ID, character or numeric allowed.
- person: person ID, a unique ID within each pedigree, numeric or character allowed.
- father: father ID, NA if no father.
- mother: mother ID, NA if no mother.
- sex: coded as 1 for male, 2 for female.
- trait: phenotype, either case-control status coded as 1 for affected and 0 for unaffected. Subjects with missing (NA) will be removed from the analysis.

Table 1: Table 1: The first 6 lines of the example pedigree file

ID	ped	person	father	mother	sex	trait
1	1	1	0	0	1	0
2	1	2	0	0	2	0
3	1	3	1	2	1	0
4	1	4	1	2	1	0

ID	ped	person	father	mother	sex	trait
5	1	5	1	2	2	0
6	1	2134	0	0	2	0

3.2 The genotype file

The genotype file is a matrix with genotypes for subjects (rows) at each variant position (columns). The first two columns are required to be named ‘ped’ and ‘person’, which are used to match subjects to their data in the pedigree data.frame. The genotypes are coded as 0, 1, 2 for autosomal markers (typically a count of the number of the minor allele).

Table 2: Table 2: The first 6 lines of the example genotype file (first 15 columns)

ped	person	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13
1	1	0	0	0	1	0	0	0	0	0	0	0	0	0
1	2	0	0	0	1	0	0	0	0	0	0	0	0	0
1	3	0	0	0	1	0	0	0	0	0	0	0	0	0
1	4	0	0	0	1	0	0	0	0	0	0	0	0	0
1	5	0	0	0	0	0	0	0	0	0	0	0	0	0
1	2134	0	0	0	0	0	1	0	0	0	0	0	0	0

3.3 The map file

The map file provides SNP positions for each SNP. The first column is required for the chromosome number, the second column is for the name of SNPs in the genotype file, and the third column is the position of SNPs in base pairs.

Table 3: Table 3: The first 6 lines of the example map file

chr	snp	pos
1	V1	498507
1	V2	498541
1	V3	498653
1	V4	498883
1	V5	499089
1	V6	499159

3.4 The covariate file (optional)

The covariate file contains covariates. The first two columns are required to be named ‘ped’ and ‘person’, which are used to match subjects to their data in the pedigree data.frame.

Table 4: Table 4: The first 6 lines of the example covariate file

ped	person	X1	X2
1	1	0	0.7172438
1	2	1	-0.3446264
1	3	1	0.6720006
1	4	1	0.2533723

ped	person	X1	X2
1	5	1	-0.2554473
1	2134	1	1.3853330

4 How to Run the Program

There are three main functions in this package which implement the statistics described in Jiang et al (2020):

1. `PedGLMM_additive_effect_model`
2. `PedGLMM_beta_smooth_only`
3. `PedGLMM_fixed_model`

After installing this `PedGLMM` R package, you can access help pages for each of these functions easily, which contain example code. For example, to access the help page for the `PedGLMM_beta_smooth_only` function, proceed as follows in R:

```
library(PedGLMM)
?PedGLMM_beta_smooth_only
```

We also provide an example `Mega2PedGLMM` function that illustrates how to use functions from the `Mega2R` R package to easily loop through genes, computing the `PedGLMM_beta_smooth_only` statistics for each gene.

4.1 Load the example data

To illustrate the functions in this package, we first load the data.

```
library(PedGLMM)
data(exampleData)
# The 'exampleData' contains four data frames: Ped, geno, cov, snpPos
ls()

## [1] "cov"      "geno"     "Ped"      "snpPos"
```

4.2 The `PedGLMM_additive_effect_model` function

This function carries out a region-based association test using our additive generalized linear mixed model (GLMM), as described in Jiang et al (2020). This statistics may not be powerful when the number of genetic variants is large.

```
add = PedGLMM_additive_effect_model(ped = Ped, geno = as.matrix(geno), covariate = as.matrix(cov))

## fixed-effect model matrix is rank deficient so dropping 39 columns / coefficients
## boundary (singular) fit: see ?isSingular
add

## $LRT
## [1] 6.076317e-14

For the case without covariates,
add_no_cov = PedGLMM_additive_effect_model(ped = Ped, geno = as.matrix(geno), covariate = NULL)

## fixed-effect model matrix is rank deficient so dropping 39 columns / coefficients
## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## unable to evaluate scaled gradient
```

```
## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## Model failed to converge: degenerate Hessian with 3 negative eigenvalues
add_no_cov
```

```
## $LRT
## [1] 3.854347e-12
```

As you can see from the examples above, the `PedGLMM_additive_effect_model` function had convergence problems. If this happens, instead of using the `PedGLMM_additive_effect_model`, one can use either `PedGLMM_beta_smooth_only` or `PedGLMM_fixed_model` instead.

4.3 The `PedGFLMM_beta_smooth_only` function

This function carries out a region-based association test using our ‘beta smooth only’ generalized functional linear mixed model (GFLMM), where the genetic effect function is assumed to be continuous/smooth. The number of basis functions used is determined by a dynamic rule as described in Jiang et al (2020). To see the function that defines this dynamic rule, look at the source code for the `dRule` function by typing `dRule` at the R prompt. This dynamic rule tries to use an appropriate number of basis functions as a function of the effective number of independent variants in the region; this ‘effective number’ is determined by an algorithm developed by Gao et al (2008).

The genetic effect function can be expanded using either B-spline or Fourier basis functions, and the order of the basis functions needs to be specified by the user.

```
order = 4

bsmooth_bsp = PedGFLMM_beta_smooth_only(ped = Ped, geno = as.matrix(geno), pos = snpPos$pos,
    order = order, covariate = as.matrix(cov), base = "bspline")
bsmooth_bsp

## $LRT
## [1] 1.19641e-06
##
## $nbetabasis
## [1] 16
##
## $M_gao
## [1] 54

bsmooth_fsp = PedGFLMM_beta_smooth_only(ped = Ped, geno = as.matrix(geno), pos = snpPos$pos,
    order = order, covariate = as.matrix(cov), base = "fspline")
bsmooth_fsp

## $LRT
## [1] 2.341872e-06
##
## $nbetabasis
## [1] 17
##
## $M_gao
## [1] 54
```

For the case without covariates,

```
bsmooth_bsp_no_cov = PedGFLMM_beta_smooth_only(ped = Ped, geno = as.matrix(geno),
    pos = snpPos$pos, order = order, covariate = NULL, base = "bspline")
bsmooth_bsp_no_cov
```

```
## $LRT
## [1] 1.699481e-05
##
## $nbetabasis
## [1] 16
##
## $M_gao
## [1] 54

bsmooth_fsp_no_cov = PedGFLMM_beta_smooth_only(ped = Ped, geno = as.matrix(geno),
  pos = snpPos$pos, order = order, covariate = NULL, base = "fspline")
bsmooth_fsp_no_cov
```

```
## $LRT
## [1] 8.540026e-05
##
## $nbetabasis
## [1] 17
##
## $M_gao
## [1] 54
```

4.4 The PedGFLMM_fixed_model function

This function carries out a region-based association test using our generalized functional linear mixed model (GFLMM). For details, see Jiang et al (2020).

The genetic variant function (GVF) and the genetic effect function can be expanded using either B-spline or Fourier basis functions, and the order of the basis functions need to be specified by the user.

```
order = 4

fixed_bsp = PedGFLMM_fixed_model(ped = Ped, geno = as.matrix(geno), pos = snpPos$pos,
  order = order, covariate = as.matrix(cov), base = "bspline")

## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, : Model is nearly unidentifiable:
## - Rescale variables?

fixed_bsp

## $LRT
## [1] 1.19641e-06
##
## $nbetabasis
## [1] 16
##
## $ngenobasis
## [1] 16
##
## $M_gao
## [1] 54

fixed_fsp = PedGFLMM_fixed_model(ped = Ped, geno = as.matrix(geno), pos = snpPos$pos,
  order = order, covariate = as.matrix(cov), base = "fspline")
fixed_fsp

## $LRT
## [1] 2.341872e-06
```

```
##
## $nbetabasis
## [1] 17
##
## $ngenobasis
## [1] 17
##
## $M_gao
## [1] 54
```

For the case without covariates,

```
order = 4

fixed_bsp_no_cov = PedGFLMM_fixed_model(ped = Ped, geno = as.matrix(geno), pos = snpPos$pos,
  order = order, covariate = NULL, base = "bspline")
fixed_bsp_no_cov

## $LRT
## [1] 1.699482e-05
##
## $nbetabasis
## [1] 16
##
## $ngenobasis
## [1] 16
##
## $M_gao
## [1] 54

fixed_fsp_no_cov = PedGFLMM_fixed_model(ped = Ped, geno = as.matrix(geno), pos = snpPos$pos,
  order = order, covariate = NULL, base = "fspline")
fixed_fsp_no_cov

## $LRT
## [1] 8.540016e-05
##
## $nbetabasis
## [1] 17
##
## $ngenobasis
## [1] 17
##
## $M_gao
## [1] 54
```

4.5 The Mega2PedGFLMM function

The `Mega2PedGFLMM` function illustrates how to use functions from the `Mega2R` R package (Baron et al, 2018) to easily loop through genes, computing the `PedGFLMM_beta_smooth_only` statistics for each gene.

It will skip genes that contain less than two polymorphic markers.

The `Mega2R` R package is designed to read a database of phenotypes and genetic data, created by the data-reformatting program `Mega2` (Baron et al, 2014), into R as a set of coordinated data frames. Once these data are nicely accessible in R, then `Mega2R` functions can be used to loop through gene regions, computing statistics for each region. By studying the code of this example `Mega2PedGFLMM` function, one can figure out

how to loop through one's own data in a similar fashion.

```
db = system.file("exdata", "seqsimGFLMM.db", package = "PedGFLMM")
ENV = init_PedGFLMM(db)
ENV$verbose = TRUE
Mega2PedGFLMM(gs = 53:54)

## tryFn() No markers in range: NM_014705, DOCK4, 7, 111366163, 111846462

## CEP104 snp24037 34 35 0
## CEP104 snp24039 69 0 0
## CEP104 snp24041 49 20 0
## CEP104 snp24048 37 30 2
## CEP104 snp24494 60 8 1
## CEP104 snp24499 56 13 0
## CEP104 snp24506 24 36 9
## CEP104 snp24507 48 18 3

## boundary (singular) fit: see ?isSingular

##   chr  gene nvariants   start      end LRT.bsm.Bsp LRT.bsm.fsp
## 1    1 CEP104          7 3728644 3773797   0.4465485   0.6334223
Mega2PedGFLMM(genes = "CEP104")

##

## 'select()' returned 1:1 mapping between keys and columns
## 'select()' returned 1:many mapping between keys and columns

## tryFn() No markers in range: 9731, CEP104, CEP104, 4283, uc031pky.1, 1, -, 3732844, 3753716

## CEP104 snp24037 34 35 0
## CEP104 snp24039 69 0 0
## CEP104 snp24041 49 20 0
## CEP104 snp24048 37 30 2
## CEP104 snp24494 60 8 1
## CEP104 snp24499 56 13 0
## CEP104 snp24506 24 36 9
## CEP104 snp24507 48 18 3

## boundary (singular) fit: see ?isSingular

##   chr  gene nvariants   start      end LRT.bsm.Bsp LRT.bsm.fsp
## 2    1 CEP104          7 3728645 3773797   0.4465485   0.6334223
```

The Mega2PedGFLMM function calls the DOPedGFLMM function for each gene region. The DOPedGFLMM function takes the data that was read into the ENV environment from the “seqsimGFLMM.db” Mega2R database and reformats as expected by the PedGFLMM_beta_smooth_only function.

5 Explanation of the Results

As shown above, our program outputs the p -value based on likelihood ratio test (LRT). The LRT is conservative and has good power performance.

6 References

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