Vignette for `MultiMed` package

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1 Overview

The `MultiMed` package implements a permutation method which adjusts for "multiple comparisons" when testing whether multiple biomarkers are mediators between a known risk factor and a disease. The approach is described in the companion paper (**?**), "Testing multiple biological mediators simultaneously." This method can significantly improve the power to detect mediators over the standard Bonferroni correction.

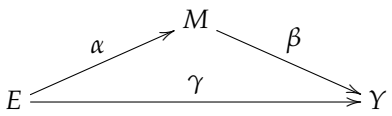
We first need to load the package:

```
> library(MultiMed)
```

2 Performing the test of mediation

The scenarios which can be considered are shown in Figure **??** for the single mediator case and Figure **??** (also shown in the (**?**) paper) for the multiple mediator case. Here, we consider simulating data where the exposure E, the mediator(s) M (or $M_i, i = 1, \ldots, K$), and the outcome Y are normally distributed. We denote by σ_E^2 the variance of E, by σ_M^2 ($\sigma_{M_i}^2$) the variance of M (M_i) conditional on E, and by σ_Y^2 the variance of Y conditional on E and M (M_i).

Figure 1: A scenario with a single possible mediator between exposure and outcome.



2.1 The `medTest` function

The function used to perform the test of mediation is `medTest`. It has seven arguments: `E`, `M`, `Y`, `Z`, `nperm`, `w`, and `useWeightsZ`. `E`, `M`, and `Y` represent matrices of size $n \times 1$, $n \times K$, and $n \times 1$, respectively, giving the exposure, mediator, and outcome values, where n is the sample size and K is the number of mediators. `E` and `Y` can also be inputted as vectors. The `Z` argument is either `NULL` or a numerical matrix having n rows. If it is not `NULL`, then the exposure, mediators, and outcome will all be initially regressed on Z, with the

residuals being used in the mediation analysis. The `nperm` argument gives the number of permutations used to estimate the null distribution, the default being 100. The `w` argument specifies whether any weighting should be done for the *E*-*M* association, as would be needed, for instance, in a scenario which considers a case-control study. The default is `w=1`, which means that all the study participants are equally weighted; `w` may also be given as a vector of length n, in which case it is first standardized to sum to 1. The `useWeightsZ` argument can be `TRUE`, in which case the weights in `w` are used for the initial regression on Z, or `FALSE`, in which case equal weights are used for this initial step.

2.2 Simulated example: Single mediator case

For a sample size of $n = 100$, we can simulate a dataset with a single mediator in the following way:

```
> set.seed(20183)
> alpha <- 0.2
> beta <- 0.2
> gamma <- 0.4
> n <- 100
> sigma2E <- 1
> sigma2M <- 1 - alpha^2
> sigma2Y <- 1 - beta^2 * (1 - alpha^2) - (alpha * beta + gamma)^2
> ## exposure:
> E <- rnorm(n, 0, sd = sqrt(sigma2E))
> ## mediator:
> M <- matrix(0, nrow = n, ncol = 1)
> M[, 1] <- rnorm(n, alpha * E, sd = sqrt(sigma2M))
> ## outcome:
> Y <- rep(0, n)
> for (subj in 1:n) Y[subj] <- rnorm(1, beta * M[subj, ], sd = sqrt(sigma2Y))
```

Note that the values of σ_E^2, σ_M^2, and σ_Y^2 were chosen so that the marginal variances of E, M, and Y are 1.

To perform a test of mediation, we use the `medTest` function. The output is a matrix with two columns: `S`, the test statistic used (the absolute value of the product of the correlations between E and M and between $r_{M|E}$ and $r_{Y|E}$, where $r_{Z_1|Z_2}$ represents the residual obtained from regressing Z_1 on Z_2) and `p`, the p-value:

```
> medTest(E, M, Y, nperm = 500)

              S     p
[1,] 0.01322964 0.546
```

2.3 Simulated example: Multiple mediator case

Now consider a scenario with $K = 10$ mediators and a sample size of $n = 100$.

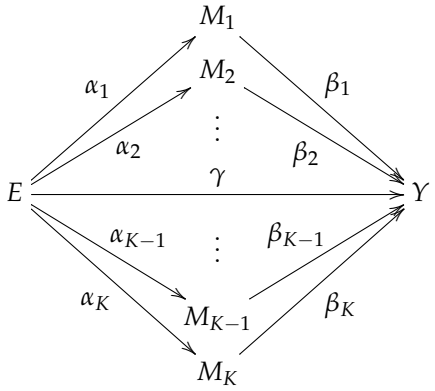
```
> set.seed(380184)
> alpha <- c(rep(0, 6), rep(0.3, 2), rep(0, 2))
> beta <- c(rep(0, 6), rep(0, 2), rep(0.3, 2))
> gamma <- 0.6
> alpha

 [1] 0.0 0.0 0.0 0.0 0.0 0.0 0.3 0.3 0.0 0.0

> beta

 [1] 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.3 0.3
```

Figure 2: A scenario with K possible mediators between exposure and outcome.




```
> n <- 100
> sigma2E <- 1
> sigma2M <- 1-alpha^2
> sigma2Y <- 1-sum(beta^2*sigma2M)-(sum(alpha*beta)+gamma)^2
> sigma2M

 [1] 1.00 1.00 1.00 1.00 1.00 1.00 0.91 0.91 1.00 1.00

> sigma2Y

[1] 0.46
```

Note that in this case `alpha` and `beta` are vectors having the i^{th} elements be α_i, respectively β_i, where $i = 1,\ldots,10$ indexes the mediators. Similarly, `sigma2M` is a vector, with the i^{th} element being $\sigma^2_{M_i}$. The values of σ^2_E, $\sigma^2_{M_i}$, and σ^2_Y were chosen so that the marginal variances of E, M_i, Y are 1.

We first simulate the data:

```
> K <- length(alpha)
> ## exposure:
> E <- rnorm(n, 0, sd = sqrt(sigma2E))
> ## mediator:
> M <- matrix(0, nrow = n, ncol = K)
> for (i in 1:K) {
+   M[, i] <- rnorm(n, alpha[i] * E, sd = sqrt(sigma2M[i]))
+ }
> ## outcome:
> Y <- rep(0, n)
> for (subj in 1:n)
+   Y[subj] <- rnorm(1, sum(beta*M[subj,])+gamma*E[subj], sd=sqrt(sigma2Y))
```

We then use the `medTest` once again to perform the test of mediation. The output is now a matrix with 10 rows, each row giving the test statistic `S` and the p-value `p` for each mediator. Note that the p-values are already implicitly considering the multiple tests being performed, so no further adjustment is necessary:

```
> medTest(E, M, Y, nperm = 500)

                 S     p
 [1,] 0.0115085655 1.000
 [2,] 0.0008037094 1.000
```

```
 [3,] 0.0009221887 1.000
 [4,] 0.0161794377 1.000
 [5,] 0.0016529532 1.000
 [6,] 0.0001764986 1.000
 [7,] 0.0343911724 0.762
 [8,] 0.0554955400 0.274
 [9,] 0.0031333508 1.000
[10,] 0.0447346023 0.484
```

2.4 Data analysis: Metabolites as mediators

We consider a data example from the (**?**) paper, using the Navy Colorectal Adenoma case-control study (**?**), with daily fish intake as the exposure of interest E and colorectal adenoma status as the outcome Y. The possible mediators are 149 serum metabolites, whose values were previously batch normalized and log transformed.

We first load the dataset:

```
> data(NavyAdenoma)
```

The first 5 columns of the `NavyAdenoma` object represent: daily fish intake, BMI, gender (coded as 0 for male, 1 for female), age, and current smoking status (coded as 0 for non-smoker, 1 for current smoker):

```
> colnames(NavyAdenoma)[1:5]

[1] "Fish"    "BMI"     "Female"  "Age"     "Smoking"
```

The next 149 columns represent the metabolite values, while the last column represents the case-control status:

```
> colnames(NavyAdenoma)[c(6:9,154)]

[1] "glycine"    "serine"     "betaine"    "alanine"    "erythritol"

> colnames(NavyAdenoma)[155]

[1] "Adenoma"

> table(NavyAdenoma$Adenoma)

  0   1
129 129
```

Due to the retrospective sampling, we consider weights incorporating the prevalence of adenoma in this age category (approximately 0.228) and the fraction of cases in the dataset for the E-M associations:

```
> prev <- 0.228
> p <- sum(NavyAdenoma$Adenoma==1)/nrow(NavyAdenoma)
> p

[1] 0.5

> w <- rep(NA, nrow(NavyAdenoma))
> w[NavyAdenoma$Adenoma == 1] <- prev/p
> w[NavyAdenoma$Adenoma == 0] <- (1-prev)/(1-p)
> table(w)

w
0.456 1.544
  129   129
```

We use `medTest` to perform the test of mediation, adjusting for the covariates BMI, gender, age, and current smoking status. As in the **?** paper, we perform this adjustment using equal weights, rather than using the weights in `w`, but users can consider using the weights in `w` both here and downstream:

```
> set.seed(840218)
> medsFish <- medTest(E=NavyAdenoma$Fish,
+                     M=NavyAdenoma[, 6:154],
+                     Y=NavyAdenoma$Adenoma,
+                     Z=NavyAdenoma[, 2:5],
+                     nperm=1000, w=w,
+                     useWeightsZ=FALSE)
```

Now find metabolite which has the lowest p-values:

```
> rownames(medsFish) <- colnames(NavyAdenoma[,-c(1:5, 154)])
> medsFish[which.min(medsFish["p"]),,drop=FALSE]
```

```
                                          S     p
docosahexaenoate (DHA; 22:6n3) 0.04989712 0.056
```

Thus, we conclude that DHA (fish oil) is a possible mediator of the association between fish intake and colorectal adenoma.