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 [Boca et al., 2014], "Testing multiple biological mediators simultaneously." This method can significantly improve the power to detect mediators over the standard Bonferroni correction.

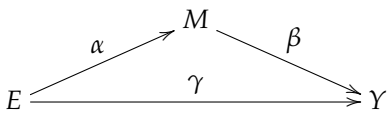
We first need to load the package:

```
> library(MultiMed)
```

2 Performing the test of mediation

The scenarios which can be considered are shown in Figure 1 for the single mediator case and Figure 2 (also shown in the [Boca et al., 2014] 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 $n = 100$.

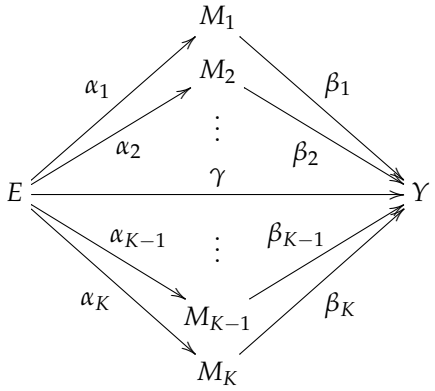
```
> set.seed(380184)
> alpha <- c(rep(0, 6), rep(0.3, 2), rep(0, 2))
> beta <- c(rep(0, 6), rep(0, 2), rep(0.3, 2))
> gamma <- 0.6
> alpha

 [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 [Boca et al., 2014] paper, using the Navy Colorectal Adenoma case-control study [Sinha et al., 1999], 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 Boca et al. [2014] 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.

References

S. M. Boca, R. Sinha, A. J. Cross, S. C. Moore, and J. N. Sampson. Testing multiple biological mediators simultaneously. *Bioinformatics*, 30(2):214–220, 2014.

R. Sinha, W. H. Chow, M. Kulldorff, J. Denobile, J. Butler, M. Garcia-Closas, R. Weil, R. N. Hoover, and N. Rothman. Well-done, grilled red meat increases the risk of colorectal adenomas. *Cancer Research*, 59 (17):4320–4324, 1999.