

Vignette for `MultiMed` package

Simina M. Boca
Innovation Center for Biomedical Informatics and
Department of Oncology, Georgetown University Medical Center
email: `smb310@georgetown.edu`,

Joshua N. Sampson
Biostatistics Branch, Division of Cancer Epidemiology and Genetics,
National Cancer Institute
email: `joshua.sampson@nih.gov`

May 19, 2021

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.

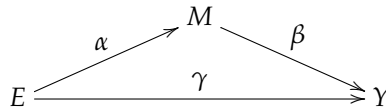
We first need to load the package:

```
> 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 $M_i, i = 1, \dots, K$), 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)
```

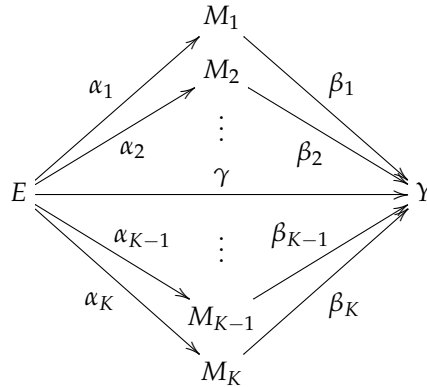
```
      S      p
[1,] 0.01322964 0.53
```

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 0.0 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 0.0 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 $i = 1, \dots, 10$ indexes the mediators. Similarly, **sigma2M** is a vector, with the i^{th} element being $\sigma_{M_i}^2$. The values of σ_E^2 , $\sigma_{M_i}^2$, and σ_Y^2 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 0.998
[5,] 0.0016529532 1.000
[6,] 0.0001764986 1.000
[7,] 0.0343911724 0.774
[8,] 0.0554955400 0.298
[9,] 0.0031333508 1.000
[10,] 0.0447346023 0.534

```

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 0.051
```

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.