Multiple Testing

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In this note I briefly review the concepts of power ant type-I error rate when conducting a single test and also in the context of multiple tests conducted simultaneously. The recommended reading for this module is Chapter 15 of the book Computer Age Statistical Inference by Efron and Hastie (2017).

(1) Power & Type-I error rate with a single test

Consider a single test for which there are two possible states of nature (H_0 or H_a) and two possible decisions (reject/do not reject, **Table 1**).

Table 1: Classification of decisions in hypothesis testing.

	Do not reject H_0	Reject H_0
H_0 holds	True Negative (N_1)	False Positive (N_2)
H_a holds	False Negative (N_3)	True positive (N_4)

Suppose we repeat our experiment a large number of times, each time re-sampling data from the population. The number of discoveries is $N_2 + N_4$. Of these, only N_4 discoveries are true. The proportion of false and true postivies are $FDP = N_2/(N_2 + N_4)$ and $TDP = N_4/(N_2 + N_4)$, these over over $N_1 + N_2 + N_3 + N_4$ trials of which $N_1 + N_2$ originated from null hypothesis and $N_3 + N_4$ were generated under H_a, respectively.

Likewise, if H_0 holds, the proportion of times we wrongly reject H_0 (type-I errors) is $N_2/(N_1 + N_2)$, and if H_A holds, the proportion of times we rightly reject H_0 is $N_4/(N_3 + N4)$. The type-I error rate and power of the experiment are the expected values of these quantities:

- Type-I error rate: $P(reject\ H_0|H_0\ holds) = E[N_2/(N_1 + N_2)]$
- Power: $P(reject\ H_0|Ha\ holds) = E[N_4/(N_3 + N_4)]$

In hypothesis testing we tune our decision rule (e.g., reject if p-values is smaller than some threshold, α) to control Type-I error rate at a low level (say $q = 0.05$). Recall that p-values are estimates of the probabilities of obtaining a test statistic as extreme or more extreme than the one we observed given that H_0 holds. Therefore, if p-values are correct and we conduct a single test each time rejecting if the p-value is smaller than 0.05 we expect a Type-I error rate of 5%.

(2) Family-Wise Error Rate (FWER)

When testing multiple hypotheses, the FWER is the probability of wrongly rejecting at least one of the null hypotheses tested.

Consider for example testing two hypotheses (H_{01} and H_{02}). Recall that the probability of the union of two events is $P(A\ or\ B) = P(A) + P(B) - P(A \cap B)$. Therefore, if H_{01} and H_{02} hold and we test each of these hypothesis with a significance level equal to α, the probability of making at least one mistake is:

$P(reject\ H_{01}\ or\ H_{02}) = P(reject\ H_{01}) + P(reject\ H_{02}) - P(reject\ H_{01}\ and\ H_{02}) = 2\alpha - P(reject\ H_{01}\ and\ H_{02}) \leq 2\alpha$ **[1]**

If both tests are independent, $P(reject\ H_{01}\ and\ H_{02}|H_{01}\ and\ H_{02}) = \alpha^2$ leading to $P(reject\ H_{01}\ or\ H_{02}|H_{01}\ and\ H_{02}) = 2\alpha - \alpha^2$.

Thus, in general, if we reject at a significance level $\tilde{\alpha} = \alpha/2$, we would be controlling the probability of making at least one mistake at a rate $\leq \alpha$.

Bonferroni correction

To keep the FWER at a level of α, the Bonferroni correction method rejects each hypothesis at a level $\tilde{\alpha} = \alpha/p$ where p is the number of hypothesis tested.

This procedure is overly conservative because, by eq. [1], the type-I error rate is expected to be lower than α, this can be particularly conservative if the test-statistics are positively correlated, that is if the probability of rejecting both tests is high.

The following example illustrate the use of Boferroni correction for two un-correlated tests.

```
 n=500
 Rsq=0 # use 0 (1) for two independent ((pefectly correlated) tests
 nRep=10000 # number of MC reps

 PVAL=matrix(nrow=nRep,ncol=2,NA)

 for(i in 1:nRep){
   x1=rnorm(n)
   x2=x1*sqrt(Rsq)+rnorm(n,sd=sqrt(1-Rsq))

   y=rnorm(n) # both nulls are true

   fm1=lm(y~x1)
   fm2=lm(y~x2)
   PVAL[i,1]=summary(fm1)$coef[2,4]
   PVAL[i,2]=summary(fm2)$coef[2,4]
 }
```

Say our target FWER is 0.1, if we do not adjust by multiple testing we have that the FWER is larger than 0.1

```
reject1=PVAL[,1]<0.1
reject2=PVAL[,2]<0.1

table(reject1,reject2)
```

```
##        reject2
## reject1 FALSE TRUE
##   FALSE  8116  881
##   TRUE    914   89
```

```
FWER=mean(reject1|reject2)
FWER
```

```
## [1] 0.1884
```

However, if we use Bonferroni's method the FWER is controled at a level below 0.1.

```
 reject1=PVAL[,1]<0.1/2
 reject2=PVAL[,2]<0.1/2
 FWER=mean(reject1|reject2)
 FWER
```

```
## [1] 0.0941
```

If we re-run the example, this time using postively correlated tests, we have that the FWER is much lower than the target FWER (0.1), this implies that our test could be overly conservative.

```
n=500
 Rsq=0.8 # use 0 (1) for two independent ((pefectly correlated) tests
 nRep=10000 # number of MC reps

 PVAL=matrix(nrow=nRep,ncol=2,NA)

 for(i in 1:nRep){
   x1=rnorm(n)
   x2=x1*sqrt(Rsq)+rnorm(n,sd=sqrt(1-Rsq))

   y=rnorm(n) # both nulls are true

   fm1=lm(y~x1)
   fm2=lm(y~x2)
   PVAL[i,1]=summary(fm1)$coef[2,4]
   PVAL[i,2]=summary(fm2)$coef[2,4]
 }

 reject1=PVAL[,1]<0.1/2
 reject2=PVAL[,2]<0.1/2
 FWER=mean(reject1|reject2)
 FWER
```

```
## [1] 0.069
```

We can think of Bonferroni's method as *adjusting p-values* by multiplying each p-value by the number of tests and then setting the adjusted-pvalues to be $pAdj = min(1, pval \times p)$ (recall, p is the number of tests); we then reject if the adjusted p-value is smaller than the target FWER (e.g., 0.05). To avoid having an overly conservative decision rule, one possiblity is to replace q in $\tilde{\alpha} = \alpha/q$ with an estimate of the number of independent tests.

Holm's method

A slighlty less conservative method (Holm's method) works as follows:

- Sort p-values from smallest to largest.
- Compare each p-value with the significance level $\tilde{\alpha}_i = \alpha/(p-i)$ (p here is the number of tests conducted, and i is the order (from sallest to largest) of the p-value).

This method is also implemented in `p.adjust(,method="holm")`.

```
pVals=c(.1,.2,.015,.01)
cbind( p.adjust(pVals,method='bonferroni') , p.adjust(pVals,method='holm'))
```

```
##      [,1]  [,2]
## [1,] 0.40 0.200
## [2,] 0.80 0.200
## [3,] 0.06 0.045
## [4,] 0.04 0.040
```

```
# Reject?
cbind( p.adjust(pVals,method='bonferroni') , p.adjust(pVals,method='holm'))<0.05
```

```
##       [,1]  [,2]
## [1,] FALSE FALSE
## [2,] FALSE FALSE
## [3,] FALSE  TRUE
## [4,]  TRUE  TRUE
```

In practice, Holm's method provides only a small power improvment relative to Bonferroni, while preserving FWER control.

(3) False discovery rate

Many modern statistical analyses requires conducting a very large number of tests. For instance, in genetic studies we may need to test the association between a phenotype and potentially millions of genetic markers (e.g., single nucleotide polymorphisms, SNPs). When the number of tests is very large, controlling Family-Wise Error Rate (i.e., targeting a very low probability of making at most one mistake) leads to overly conservative tests, thus reducing power. Thus, an alternative is to use a decision rule for rejection that control the expected proportion of mistakes among the discoveries.

Using the numbers in **Table 1** we can define the false discovery proportion and its expected value as follows

- **False Discovery Proportion** is $FDP = N_2/(N_2 + N_4)$, and the
- **False Discovery Rate (FDR)** is the expected value of the FDP over conceptual repeated sampling, that is $FDR = E[N_2/(N_2 + N_4)]$.

For any given decision rule and data we can obsreve the total number of rejections $N_2 + N_4$; however, at first glance, estimating how many of these are likely to be rejections of true nulls (N_2) is not straightoforward. However, a simple procedure by Benjaminy and Hocberg (1995) can be used to define a decision rule with adequate FDR control.

Benjamini-Hocberg (BH) procedure:

- Sort the p-values from smallest to highest.
- For the sorted p-vaues compute $[i/p] \times \alpha$, where i is the order of the p-value ($i = 1$ for the smallest p-value), p is the number of tests conducted and α is the FDR-threshold ($\alpha \in [0, 1]$).
- Reject if $pValue[i] < [i/p] \times \alpha$.

If all the tests conducted are independent and all originate from null hypothesis, the BH procedure controls FDR at the desired level (α); in practice some proportion (typically a very small one when the number of hypothesis tested is very large) of the tests originate from alternative hypothesis. If the proportion of tests that originates from null hypothesis is π_0 and tests are indpeendent, the BH procedure controls FDR at $\pi_0\alpha < \alpha$. The function `p.adjust(,method='fdr')` adjust p-values using the BH procedure, after adjustment we simply reject for all adjuste p-values $< \alpha$ (e.g., $q = 0.05$). The following example illustrates the use of the BH procedure, in the example 5% of the hypothesis originate from H_a's.

```
pH0=0.95
nTests=5000
n=1000 # sample size
pVals=rep(NA,nTests)
isHA=runif(nTests)>pH0
varB=.03 #  variance explained if Ha holds

for(i in 1:nTests){
  x=rnorm(n)
  y=rnorm(n)
  if(isHA[i]){
    y=y+x*rnorm(1,sd=sqrt(varB)) # adding an effect if Ha
  }
  pVals[i]=summary(lm(y~x))$coef[2,4]
}

pADJ.Bonf=p.adjust(pVals,method='bonferroni')
pADJ.Holm=p.adjust(pVals,method='holm')
pADJ.FDR=p.adjust(pVals,method='fdr')

# number of rejections by method
sum(pADJ.Bonf<0.05)
```

```
## [1] 105
```

```
sum(pADJ.Holm<0.05)
```

```
## [1] 105
```

```
sum(pADJ.FDR<0.05)
```

```
## [1] 154
```

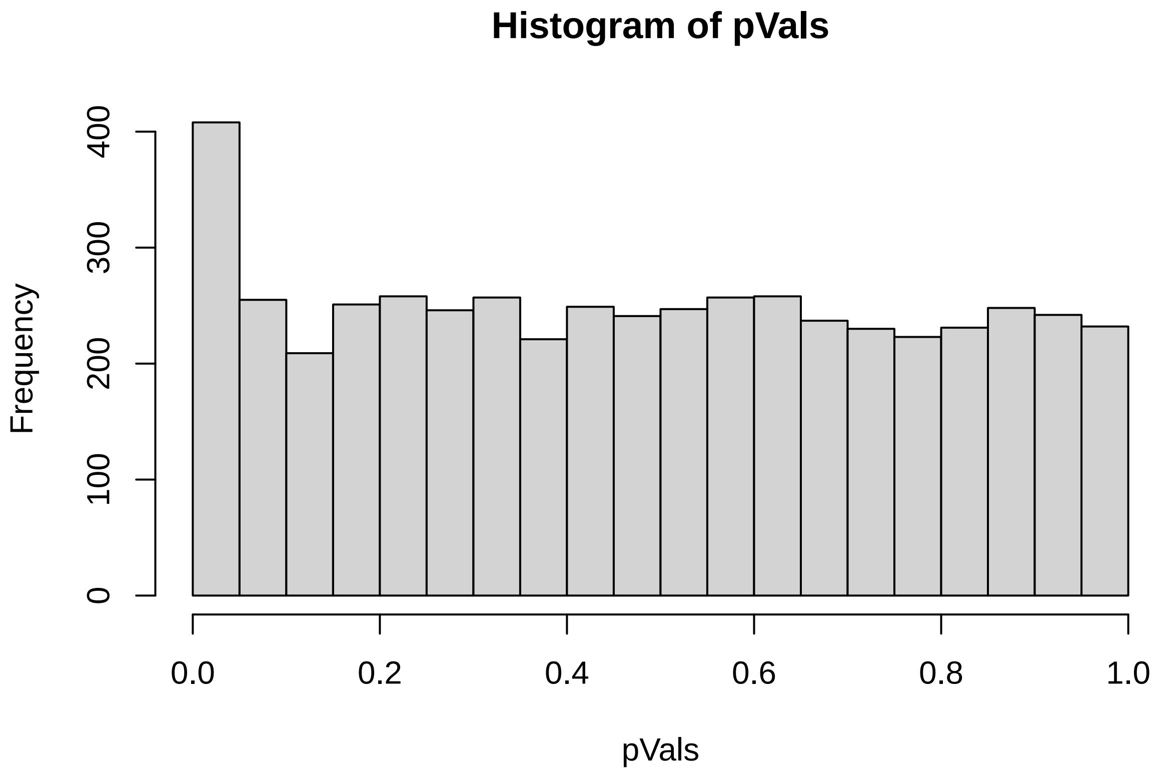
If we use a FDR for rejection equal to 0.05, then the false discovery proportion in the single monte carlo replicate was

```
mean((pADJ.FDR<0.05)[!isHA])
```

```
## [1] 0.00210615
```

Recall that under the null hypothesis, the distribution of the p-values is uniform. What is the empirical distribution of the p-values when some proprotion of the tests originate from alternative hypotheses? Instead of uniform in this case we see an increase in the frequency of low-pvalues, the majority of which originate from alternative hypothesis.

```
hist(pVals,30)
```


Histogram of pVals
Frequency
pVals