

# ADVANCED BIOSTATISTICS

## ABSTAT18

### Multiple Testing Issues

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IGC, April 3rd - 6th, 2018

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- $V$ : r.v. which represents the number of false positive genes among  $m$  (multiple hypotheses),  
 $P(V \geq 1) = 1 - P(V = 0) = 1 - (1 - \alpha)^m$ ,  
where  $\alpha$  is the probability of rejecting the null hypothesis when it is true: (*Type I Error*),  $P(\text{Rej } H_0 | H_0 \text{ True})$ .

Number of hypothesis tested ( $m$ )	False positives incidence ( $m \times \alpha$ )	Probability of 1 or more false positives by chance ( $1 - (1 - 0.05)^m$ )
1	$1/20 = 0.05$	0.050
2	$2 \times (1/20) = 0.1$	0.098
20	$20 \times (1/20) = 1$	0.642
100	$100 \times (1/20) = 5$	0.994

**Problem:** When many hypotheses are tested, the probability of a type I error (false positive) increases sharply with the number of hypotheses.

## Types of error control

- ▶ Let  $H_{01}, H_{02}, \dots, H_{0m}$  denote the null hypotheses corresponding to the  $m$  tests.
- ▶ Suppose  $m_0$  null hypotheses are true and  $m_1$  null hypotheses are false.
- ▶ Let  $c$  denote a value between 0 and 1 that will serve as a cutoff for significance:
  - ▶ Reject  $H_{0i}$  if  $p_i \leq c$  (declare significant difference in the expression levels)
  - ▶ Do not reject  $H_{0i}$  if  $p_i > c$  (declare non-significant difference in the expression levels)

	Rejected $H_0$	Not Rejected $H_0$	
True $H_0$	$V$	$U$	$m_0$
False $H_0$	$S$	$T$	$m_1$
	$R$	$m - R$	$m$

$V$ : false positives (type I error)

$T$ : false negatives (type II error)

- ▶ Suppose one test of interest has been conducted for each of  $m$  genes in a microarray experiment.
- ▶ Let  $p_1, p_2, \dots, p_m$  denote the p-values corresponding to the  $m$  tests.

- ▶ **PCER:** Per-comparison error rate, the expected value of the number of Type I errors over the number of hypotheses,  $PCER = \frac{E(V)}{m}$ .
- ▶ **PFER:** Per-family error rate, the expected number of Type I errors,  $PFER = E(V)$ .
- ▶ **FWER:** Family-wise error rate: the probability of at least one type I error,  $FWER = P(V \geq 1)$ .
- ▶ **FDR:** False discovery rate, is the expected proportion of incorrectly rejected null hypotheses,  $FDR = \frac{E(V)}{R}$  for  $R > 0$  (number of rejected null hypotheses).

## FWER – Family-Wise Error Rate

- ▶ Many procedures have been developed to control the Family-Wise Error Rate (the probability of at least one type I error):  $P(V \geq 1)$
- ▶ Two general types of FWER corrections:
  1. Single Step: equivalent adjustments made to each p-value.
  2. Sequential: adaptive adjustment made to each p-value.

- └ FWER – Family-Wise Error Rate
  - └ Single-step approach: Bonferroni

## Single-step approach: Bonferroni

- ▶ The Bonferroni's Method is the simplest way to achieve control of the FWER at any desired level  $\alpha$ .
- ▶ Simply choose  $c = \alpha/m$ .
- ▶ With this value of  $c$ , the FWER will be no larger than  $\alpha$  for any family of  $m$  tests.



- └ FWER – Family-Wise Error Rate
  - └ Sequential Adjustments: Holm's method

## Sequential Adjustments: Holm's method

- ▶ Let  $p_{(1)}, p_{(2)}, \dots, p_{(m)}$  denote the  $m$   $p$ -values ordered from smallest to largest.
- ▶ Find the **largest** integer  $k$  so that:

$$p_{(i)} \leq \frac{\alpha}{m-i+1} \text{ for all } i = 1, \dots, k.$$

- └ FWER – Family-Wise Error Rate
- └ Sequential Adjustments: Holm's method

## Some considerations

- ▶ FWER is appropriate when you want to guard against ANY false positives.
- ▶ FWER criteria may be too restrictive because control of false positives implies a considerable increase of false negatives.
- ▶ FWER is too conservative because it depends on the overall number of tests ( $m$ ).
- ▶ Holm's method is less conservative than the Bonferroni's Method.

## FDR – False Discovery Rate

In practice, however, many biologists seem willing to accept that some errors will occur, as long as this allows findings to be made. For example a researcher might consider acceptable a small proportion of errors (say 10%, 20%) between her findings. In this case, the researcher is expressing interest in controlling the **false discovery rate** (FDR).

	Rejected $H_0$	Not Rejected $H_0$	
True $H_0$	$V$	$U$	$m_0$
False $H_0$	$S$	$T$	$m_1$
	$R$	$m - R$	$m$

$V$ : false positives (type I error)

$T$ : false negatives (type II error)

- ▶ FDR is designed to control the proportion of false positives among the set of rejected hypothesis ( $R$ ).
- ▶ 
$$\text{FDR} = \frac{E(V)}{R}$$

- ▶ FDR is the proportion of false positives among all the genes initially identified as being differentially expressed.
- ▶ If one obtains a list of differentially expressed genes where the FDR is controlled at, say, 20%, one will expect that a 20% of these genes will represent false positive results.

- └ FDR – False Discovery Rate
  - └ Benjamini and Hochberg FDR

## The Benjamini and Hochberg Procedure for Strongly Controlling FDR at Level $\alpha$

- ▶ Let  $p_{(1)}, p_{(2)}, \dots, p_{(m)}$  denote the  $m$   $p$ -values ordered from smallest to largest.
- ▶ Find the **largest integer**  $k$  so that  $p_{(k)} \leq \frac{k \times \alpha}{m}$ .

This correction is the least stringent of all previous options, and therefore tolerates more false positives.

There will be also less false negative genes.

## Adjust p-values in R – `p.adjust(p,method)`

Possible methods:

"holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none"

Suppose we are testing 10 hypotheses resulting in the following p-values:

```
p<-c(0.001,0.002,0.006,0.013,0.024,0.168,0.231,0.254,0.319,0.56)
```

Apply `p.adjust` function according to Benferroni, Holm's and BH methods:

```
p.adjust(p, "bonferroni");p.adjust(p, "holm");p.adjust(p, "BH")
```

```
[1] 0.01 0.02 0.06 0.13 0.24 1.00 1.00 1.00 1.00 1.00
```

```
[1] 0.01 0.018 0.048 0.091 0.144 0.840 0.924 0.924 0.924 0.924
```

```
[1] 0.01 0.01 0.02 0.032 0.0480 0.280 0.317 0.317 0.354 0.560
```

## Some final considerations

### FWER vs FDR

- ▶ The decision of controlling FDR or FWER depends on the goals of the experiment.
- ▶ If the objective is *gene fishing*, allowing a certain number of false positives to be reasonable, then FDR is preferable.
- ▶ If instead one is working with a shorter number of hypotheses, in which we want to verify if some specific ones are significant, then FWER is the appropriate criteria.
- ▶ FDRs are more appropriate in large sets of hypotheses.



## Remarks

- ▶ Which multiple tests correction should be used? As long as the conditions you have for the data meet with the assumptions in particular multiple tests corrections, use the one that gives the highest power. **Using an FDR method is common these days.**
- ▶ 5% (or 95% confidence) is a convention, not a magic number (same to 1% or 0.1%). If you do not have any particular reason to favour a particular threshold, use a convention.

## Acknowledgements:

Antónia Turkman (DEIO – FCUL) and Carina Silva-Fortes (ESTeSL – IPL), for allowing the use of some material produced by us in previous courses.

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