

# Statistics primer: Hypothesis testing

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# Foreword

*the function of significance tests is to prevent you from making a fool of yourself, and not to make unpublishable results publishable [1]*

at the end of the course, we will understand

*Why Most Published Research Findings Are False. [3]*

and will pledge to not make fools of ourselves too frequently.

# Statistical hypothesis testing

- ▶ Testing a working hypothesis
- ▶ Contrast an observed result to a *comparable* random distribution (assumptions!). If it is *different enough* from what we would expect by chance, then we might have an interesting result.

# The testing process

1. Research hypothesis, as defined by our experimental design
2. State the *null hypothesis*  $H_0$  (no difference with random distribution) and *alternative hypothesis*  $H_1$  (there is a difference with random distribution)
3. Consider statistical assumptions and decide which test is appropriate.
4. Perform test and carefully interpret results (see below)

# Choosing a test statistic

- ▶ Continuous data (such as micro-array, quantitative proteomic, ...) and we want to compare means: *t-test*
- ▶ Count data (high-throughput sequencing) and we want to compare the number of reads between two conditions: *negative binomial*
- ▶ Gene set enrichment (is there an enrichment of genes with a specific function in my set of interesting genes): *hypergeometric*

Check what is used in the literature!

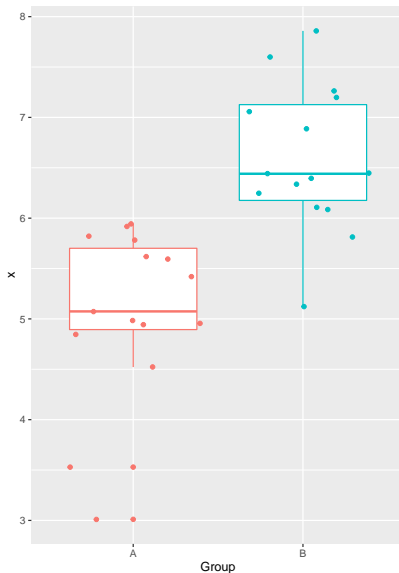
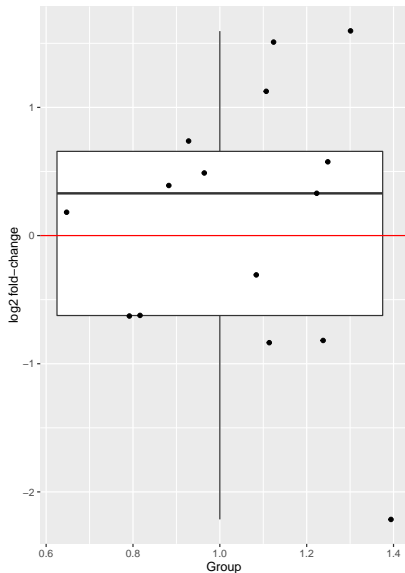
# Type of tests: (non-)parametric

- ▶ Parametric: assumption is that the data comes from a population that follows a probability distribution
- ▶ Non-parametric: no defined/fixed parameters

When assumptions are correct, a parametric test has more *statistical power*.

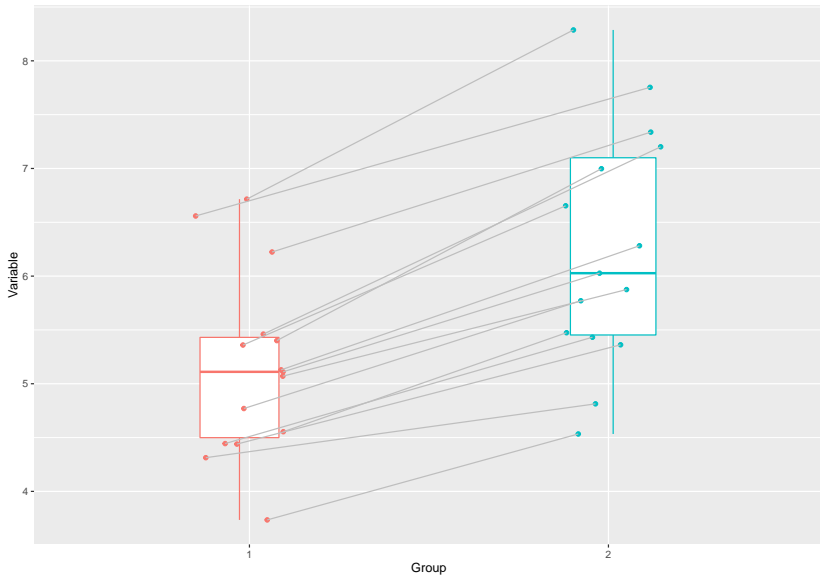
In practice, as most studies are under-powered, non-parametric tests are not an option.

# Types of tests: One-sample or two-sample tests



# Types of tests: paired tests

Measurements in two conditions are paired.





# Working example: Student's t-test

Comparing means from 2 groups, continuous data.

- ▶  $H_0 : \mu_1 = \mu_2$
- ▶  $H_1 : \mu_1 \neq \mu_2$

Assumptions:

- ▶ data is normally distributed
- ▶ data are independent and identically distributed
- ▶ equal or un-equal (Welch test) variance
- ▶ t-test is robust to deviations.
- ▶ (All models are wrong. Some are useful)

# Welch test (t-test with unequal variances)

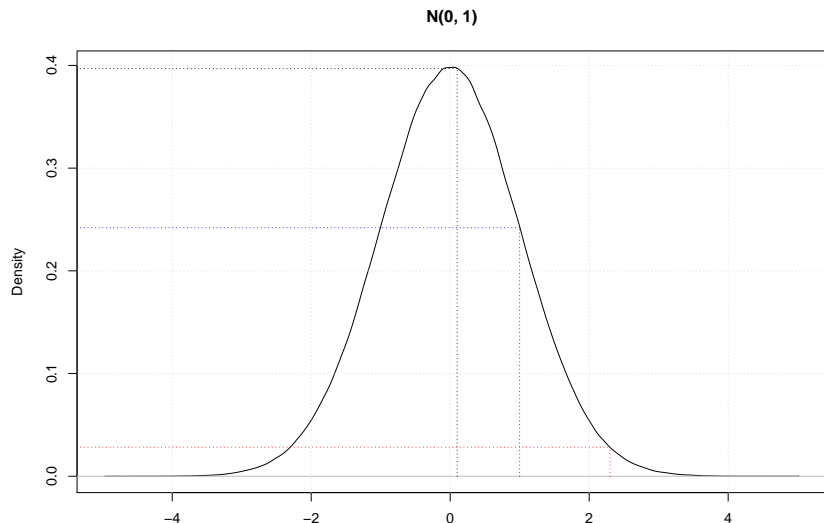
Two samples of sizes  $n_1$  and  $n_2$

$$t = \frac{\bar{X}_1 - \bar{X}_2}{s_{\bar{X}_1 - \bar{X}_2}}$$

where

$$s_{\bar{X}_1 - \bar{X}_2} = \sqrt{\frac{s_{x1}^2}{n_1} + \frac{s_{x2}^2}{n_2}}$$

# How to we estimate how different we are from *random*



**p-value:** how (un)likely it would be to observe a value as extreme or more extreme under  $H_0$ .

# P-value

**p-value:** how (un)likely it would be to observe a value as extreme or more extreme under  $H_0$ .

If p-value  $\leq$  than (arbitrary) significance level  $\alpha$ , then we reject  $H_0$ .

# What can go wrong

	H0 is true	H0 is false
H0 is rejected	Type I error, FP	correct TP
H0 not rejected	correct TN	Type II error, FN

power of a test =  $1 - \text{type II}$

(FP: false positive, TP: true positive, FN: false negative, TN: true positive)

**False discovery rate:**  $\frac{FP}{FP+TP}$

# In R

```
set.seed(1)
x <- rnorm(15, mean = 7, sd = 1)
y <- rnorm(15, mean = 5, sd = 1.2)
t.test(x, y)
```

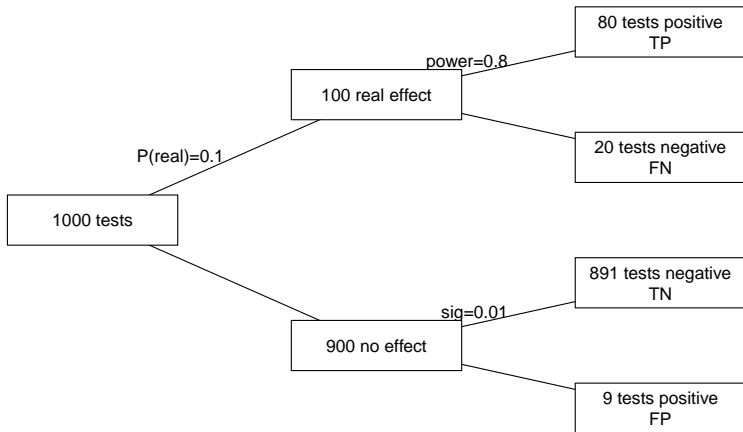
```
##
##  Welch Two Sample t-test
##
## data:  x and y
## t = 5.4214, df = 27.998, p-value = 8.778e-06
## alternative hypothesis: true difference in means is not
## 95 percent confidence interval:
##  1.259224 2.788685
## sample estimates:
## mean of x mean of y
##  7.100843  5.076888
```

# But

Is the p-value really what we want? What is the probability that we make a fool of ourselves?

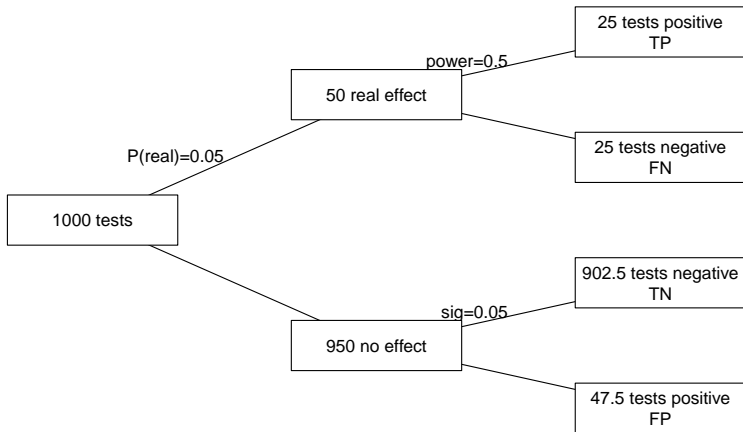
What about the power of our test? Let's calculate a false positive rate.

False discovery rate = 0.101

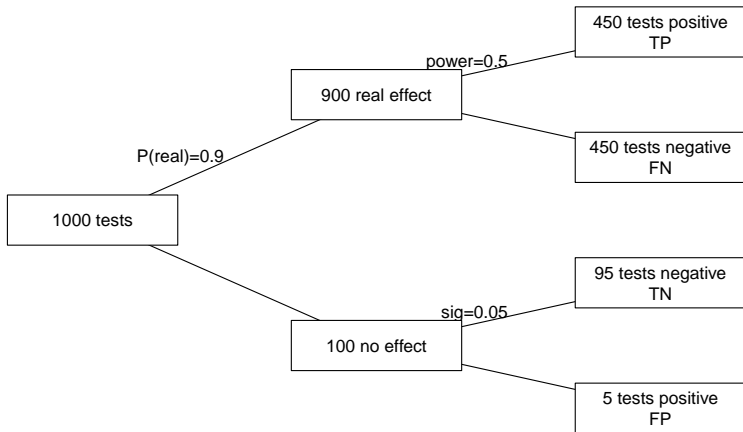


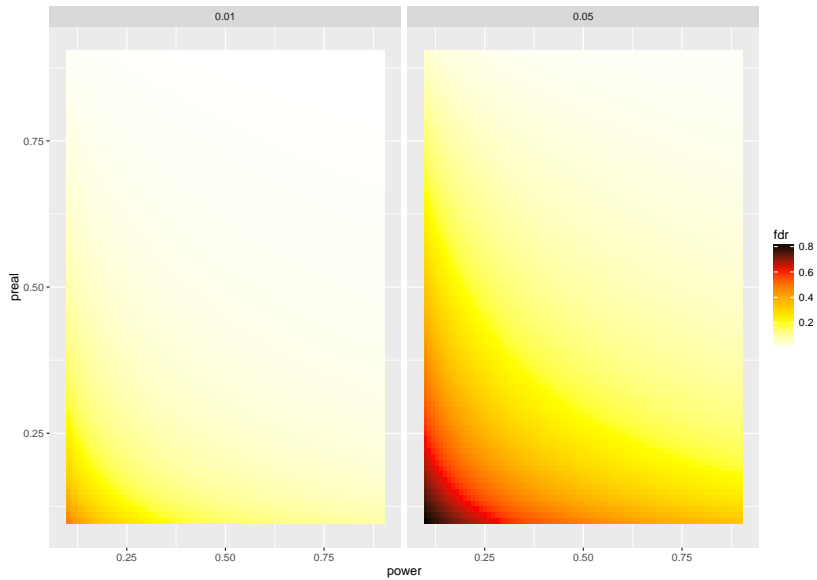


False discovery rate = 0.655



False discovery rate = 0.011





# Adjustment for multiple testing

# References

- [1] David Colquhoun *An investigation of the false discovery rate and the misinterpretation of p-values* R. Soc. open sci. 2014 1 140216; doi:10.1098/rsos.140216.
- [2] Regina Nuzzo *Scientific method: Statistical errors* 2014 Nature 506, 150–152 doi:10.1038/506150a
- [3] Ioannidis JPA *Why Most Published Research Findings Are False.* 2015 PLoS Med 2(8): e124. doi:10.1371/journal.pmed.0020124.