Hypothesis Testing

Wolfgang Huber

Aims for this lecture

Understand the basic principles of decision making by hypothesis testing, pitfalls, strengths, use cases and limitations

What changes when we go from single to multiple testing?

- false discovery rates
- p-values
- multiple testing ‘adjustments’
- hypothesis filtering and weighting

How to make rational decisions based on noisy, finite data?

Examples:

- Testing efficacy of a drug on people
  - lack of complete experimental control
  - finite sample size
- Effect of a fertilizer, a genetic variant, … on phenotype of plants / animals in an outdoors field trial
- Lady testing tea, clairvoyant, telepath, …
- Toxicology

+: No understanding of mechanism involved / needed / desired

-: Wouldn't we *want* to use any available understanding or 'priors'?

The fundamental tradeoff of statistical decision making

←bias accuracy→

dispersion→

↓ precision

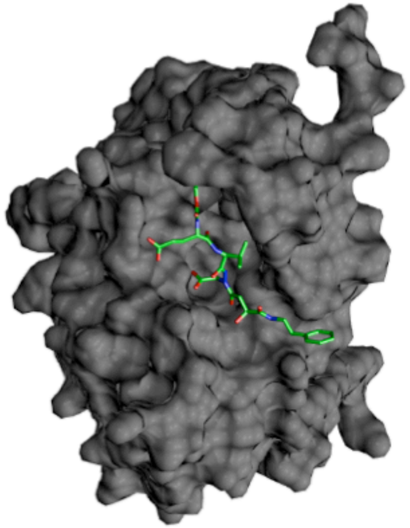
Comes in various guises

Accuracy vs Precision

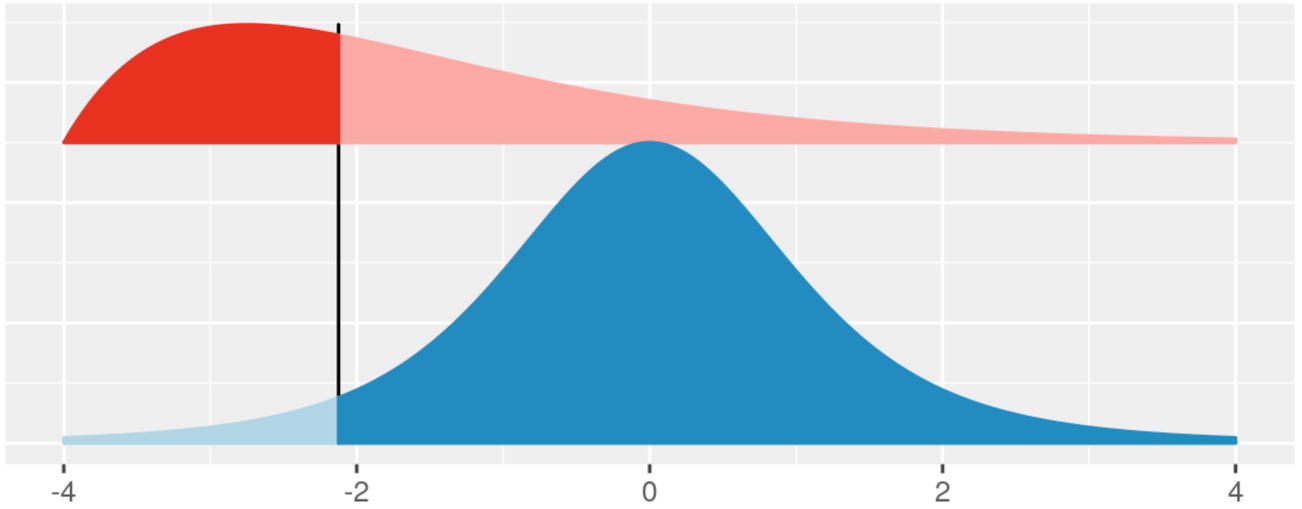
Bias vs Variance

Model complexity vs overfitting

Basic problem: binary decision

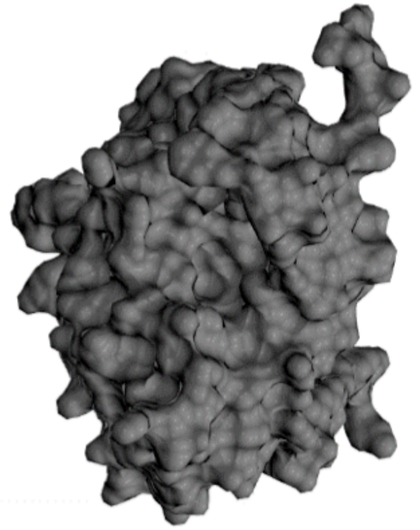


ligand binds (better than the competitors)

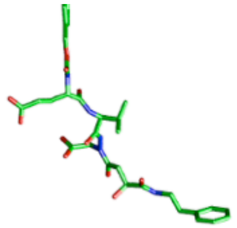


some useful number *x* computed from the data

True Positive | False Negative | False Positive | True Negative



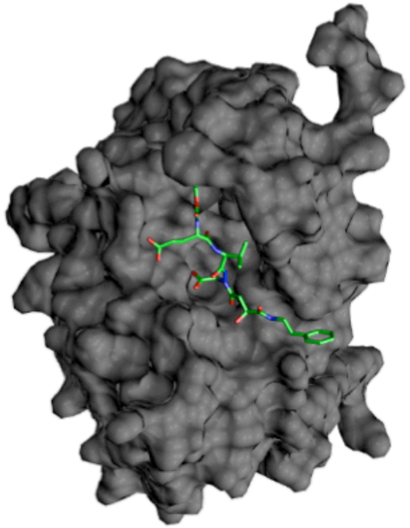
ligand does not bind (or worse than the competitors)



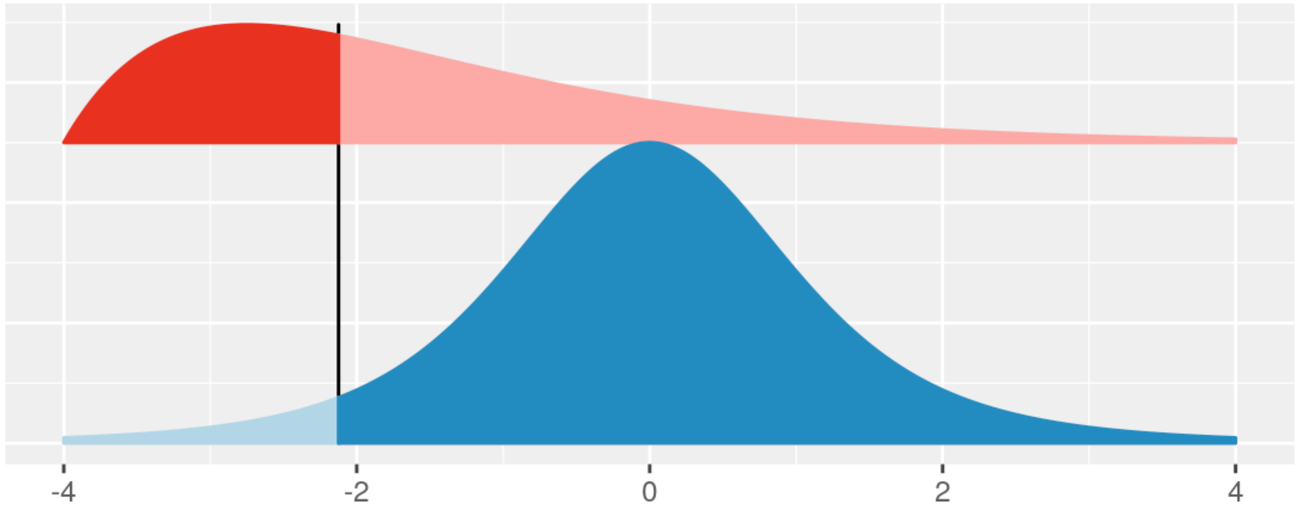
False discovery rate

$$\mathbf{FDR} = \frac{\textbf{area shaded in light blue}}{\textbf{sum of the areas left of the vertical bar (light blue + strong red)}}$$

Basic problem: binary decision

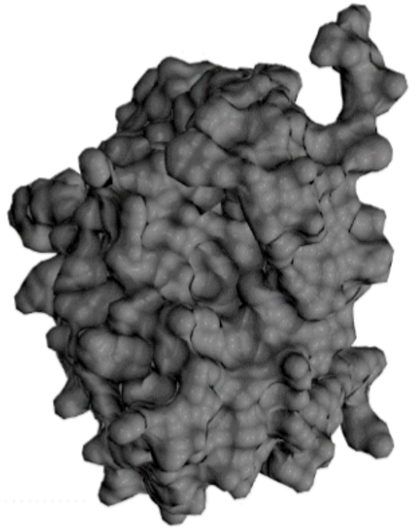


ligand binds (better than the competitors)

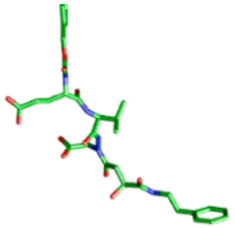


some useful number *x* computed from the data

True Positive | False Negative | False Positive | True Negative



ligand does not bind (or worse than the competitors)

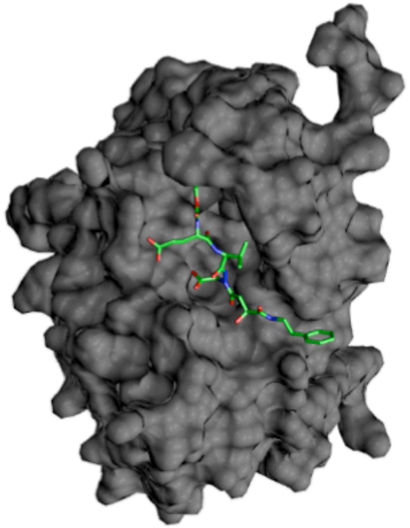


False discovery rate $$\mathbf{FDR} = \frac{\textbf{area shaded in light blue}}{\textbf{sum of the areas left of the vertical bar (light blue + strong red)}}$$

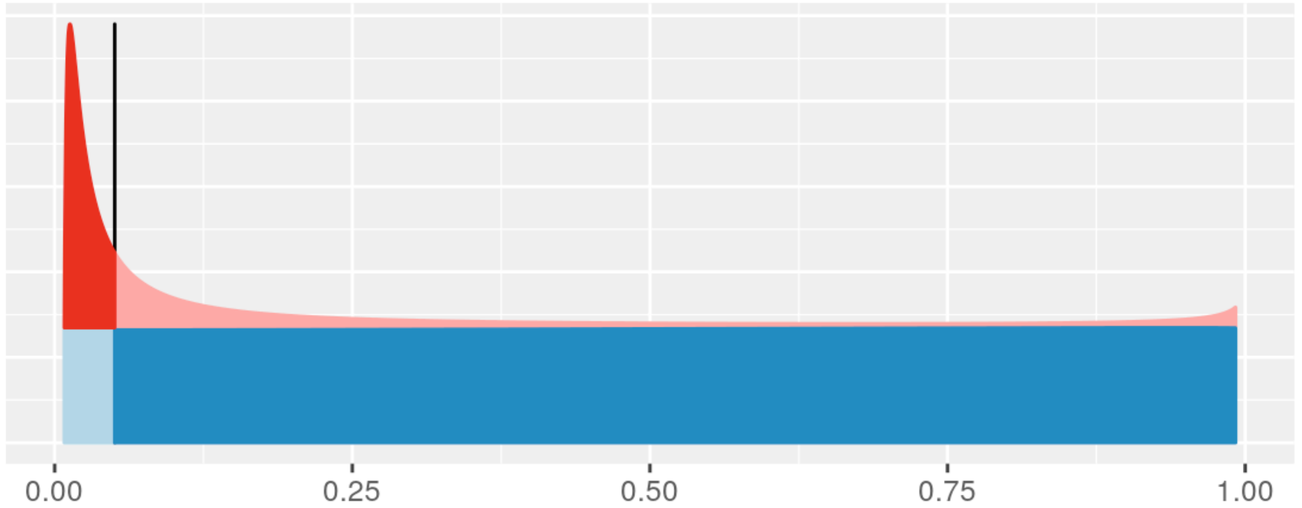
For this, we need to know:

1. the distribution of *x* in the blue class (the blue curve),
2. the distribution of *x* in the red class (the red curve),
3. the relative sizes of the blue and the red classes.

Basic problem: binary decision



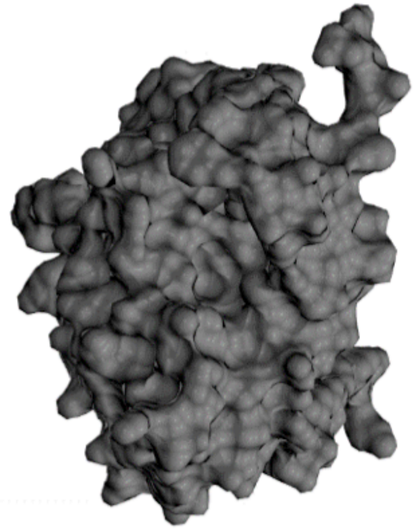
ligand binds (better than the competitors)



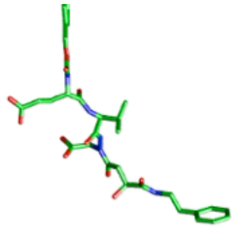
another useful number computed from the data: *p*

True Positive | False Negative | False Positive | True Negative

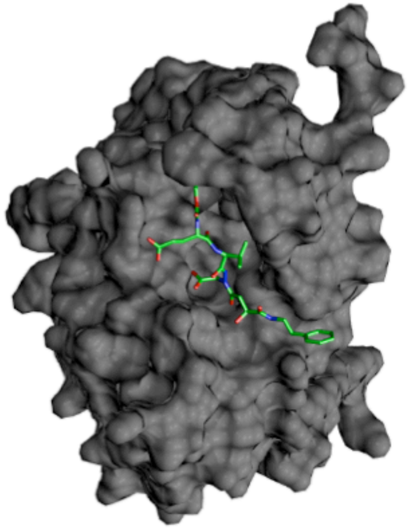
$$\text{p-value} = \frac{\text{area shaded in light blue}}{\text{overall blue area}}$$



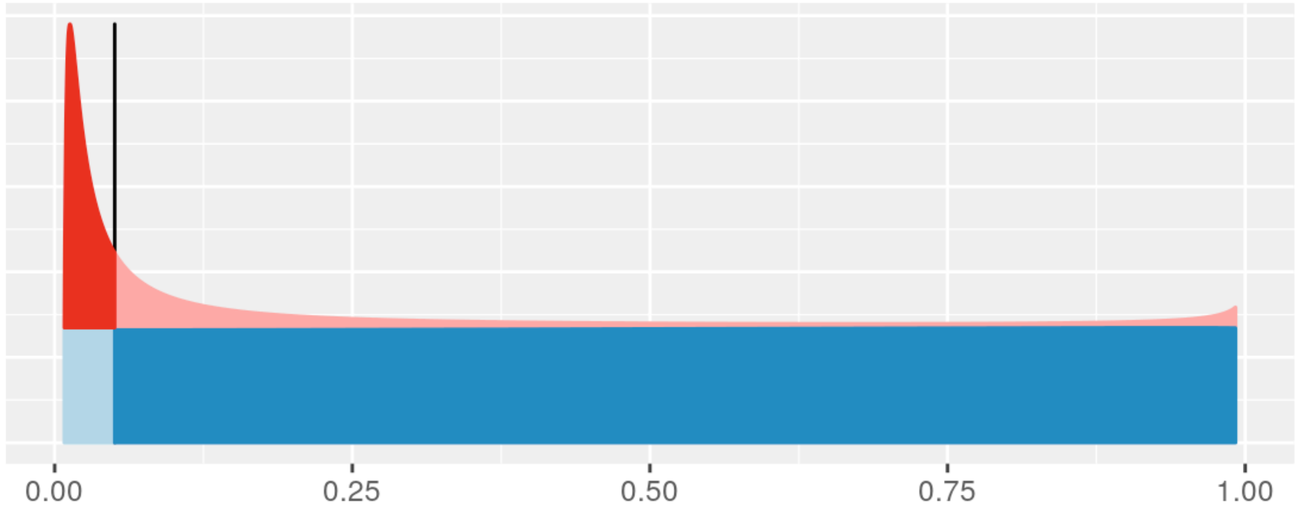
ligand does not bind (or worse than the competitors)



Basic problem: binary decision

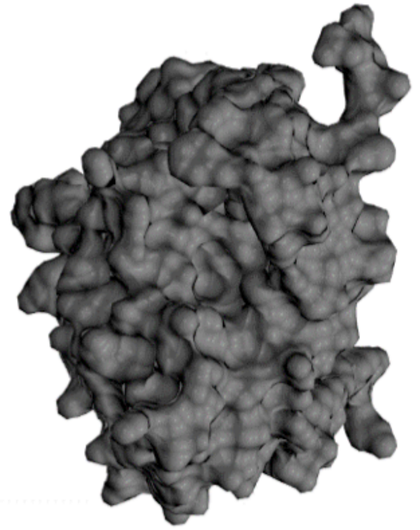


ligand binds (better than the competitors)

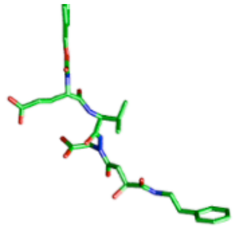


another useful number computed from the data: *p*

True Positive | False Negative | False Positive | True Negative



ligand does not bind (or worse than the competitors)

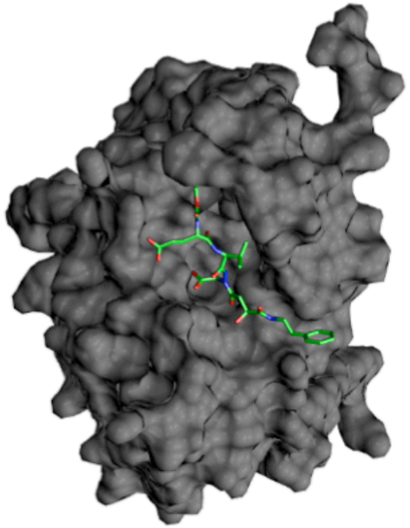


$$\text{p-value} = \frac{\text{area shaded in light blue}}{\text{overall blue area}}$$

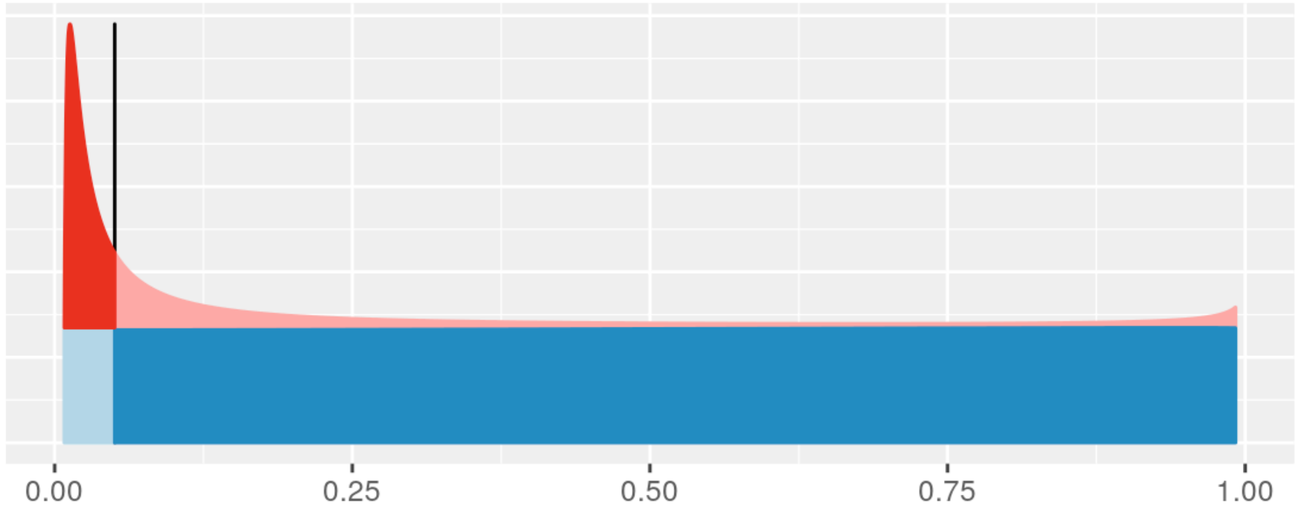
For this, we need to know:

1. the distribution of *x* in the blue class ("null hypothesis").
2. ~~the distribution of *x* in the red class (the red curve),~~
3. ~~the relative sizes of the blue and the red classes.~~

Basic problem: binary decision

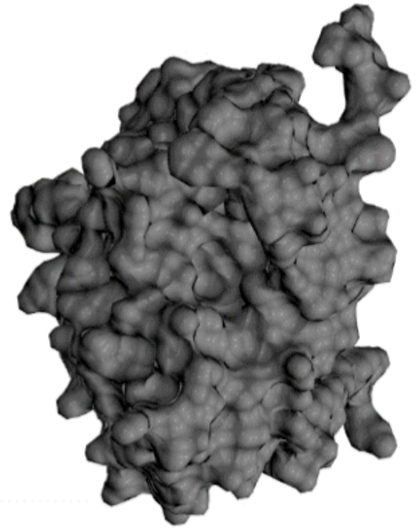


ligand binds (better than the competitors)

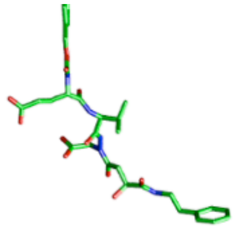


another useful number computed from the data: *p*

True Positive | False Negative | False Positive | True Negative



ligand does not bind (or worse than the competitors)



$$\text{p-value} = \frac{\text{area shaded in light blue}}{\text{overall blue area}}$$

For this, we need to know:

1. the distribution of *x* in the blue class ("null hypothesis").
2. ~~the distribution of *x* in the red class (the red curve),~~
3. ~~the relative sizes of the blue and the red classes.~~

What could possibly go wrong? What's the difference between p-value and FDR?

Machine Learning

Lots of free parameters

Lots of training data

Using multiple variables

... or objects that are not even 'variables' (e.g. images)

Hypothesis testing

Some theory/model and no or few parameters

No training data

More rigid/formulaic

Regulatory use

Machine Learning

Lots of free parameters

Lots of training data

Using multiple variables

... or objects that are not even 'variables' (e.g. images)

Hypothesis testing

Some theory/model and no or few parameters

No training data

More rigid/formulaic

Regulatory use

Example

Toss a coin a number of times ⇒

If the coin is fair, then heads should appear half of the time (roughly).

But what is “roughly”? We use combinatorics / probability theory to quantify this.

Suppose we flipped the coin 100 times and got 59 heads. Is this ‘significant’?

Binomial distribution

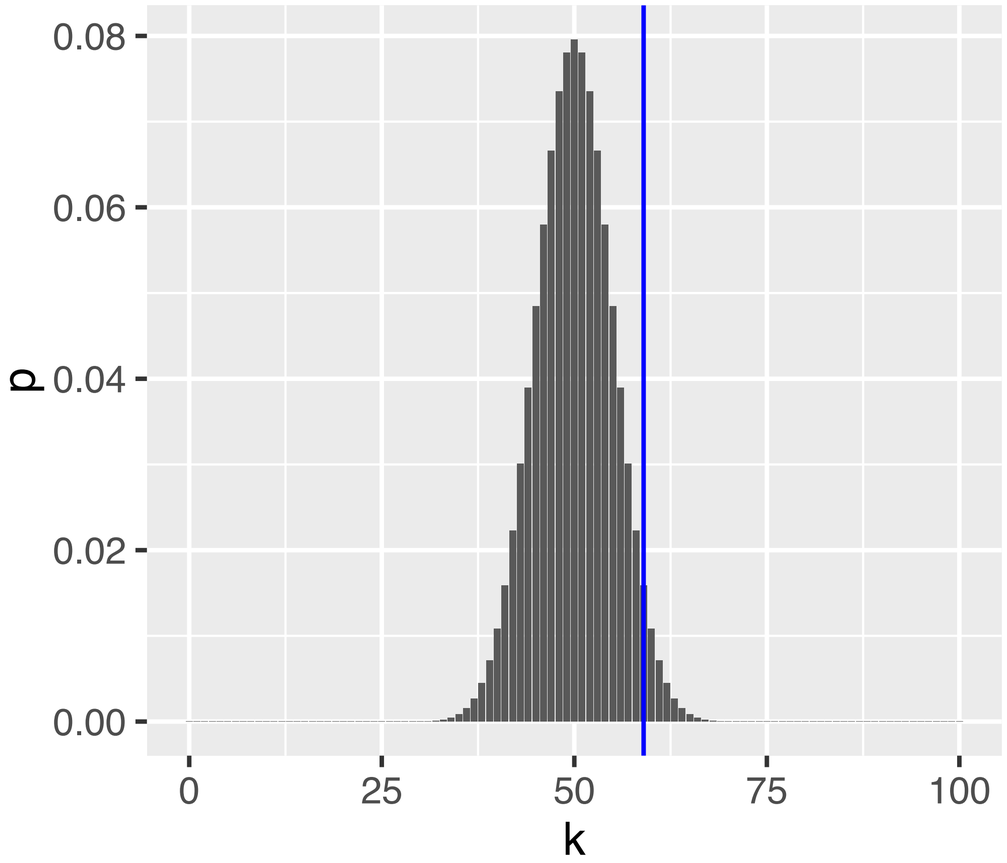


Figure 6.3: The binomial distribution for the parameters $n = 100$ and $p = 0.5$,

$$P(K = k \mid n, p) = \left(\begin{array}{c} n \\ k \end{array} \right) p^k \, (1-p)^{n-k}$$

Rejection region

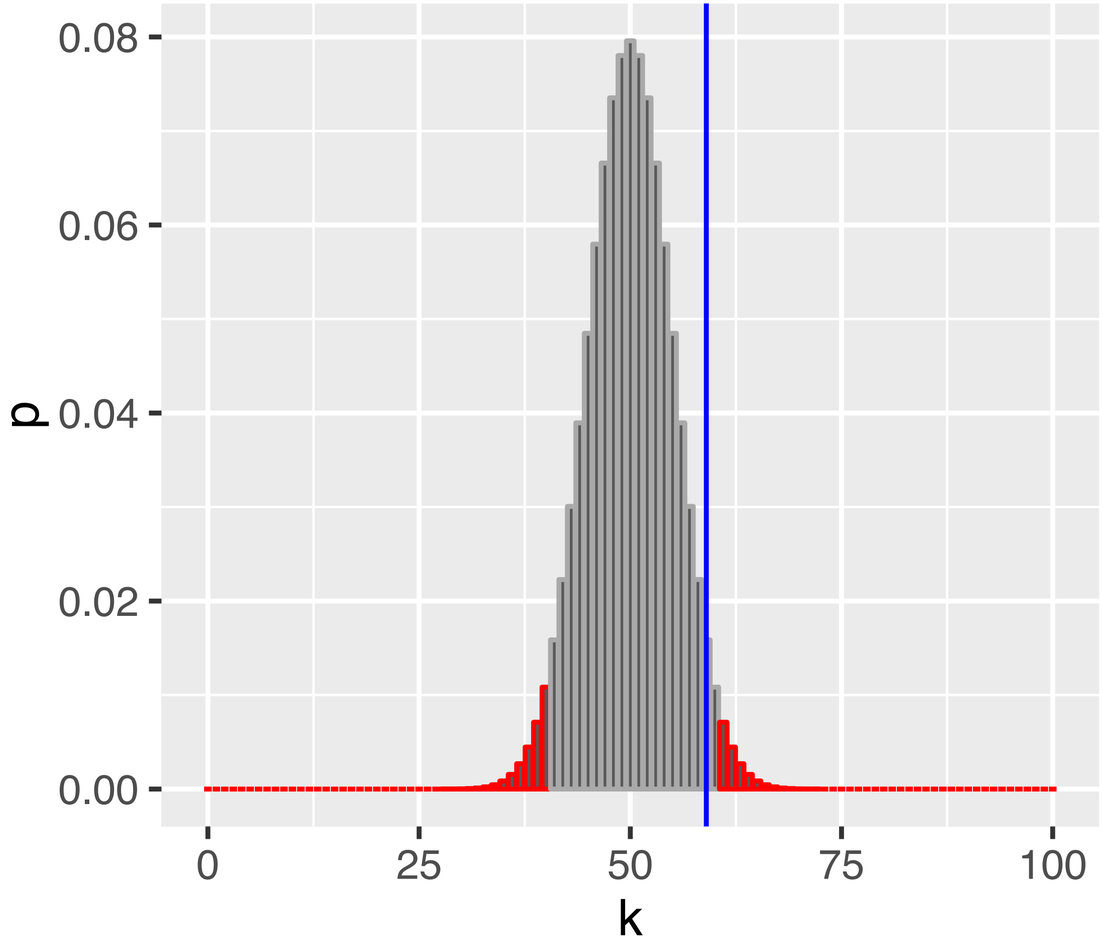


Figure 6.5: As Figure 6.3, with rejection region (red) whose total area is $\alpha = 0.05$.

Questions

- Does the fact that we don't reject the null hypothesis mean that the coin is fair?
- Would we have a better chance of detecting an unfair coin if we did more coin tosses? How many?
- If we repeated the whole procedure and again tossed the coin 100 times, might we **then** reject the null hypothesis?
- Our rejection region is asymmetric - its left part ends with 40, while its right part starts with 61. Why is that? Which other ways of defining the rejection region might be useful?

The Five Steps of Hypothesis Testing

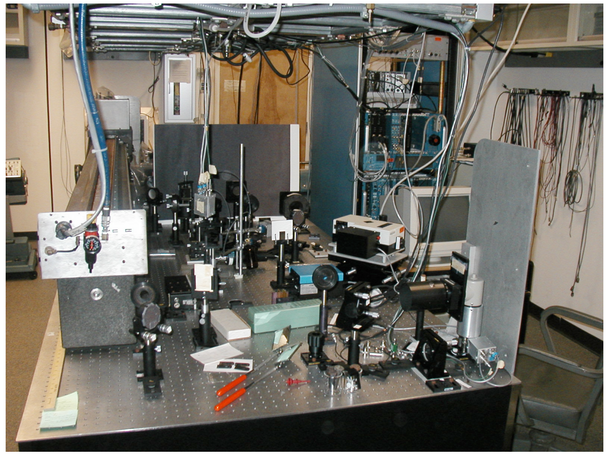
Choose an experimental design and a data summary function for the effect that you are interested in: the **test statistic**

Set up a **null hypothesis**: a simple, computationally tractable model of reality that lets you compute the null distribution of the test statistic, i.e. all its possible outcomes and each of their probabilities.

Decide on the **rejection region**, i.e., a subset of possible outcomes whose total probability is small (**significance level**).

Do the experiment, collect data,
compute the test statistic.

Make a **decision**: reject null hypothesis
if the test statistic is in the rejection region.



The Five Steps of Hypothesis Testing

Choose an experimental design and a data summary function for the effect that you are interested in:

Set up a **null hypothesis**: ...eality that lets you compute the ... possible outcomes and ea...

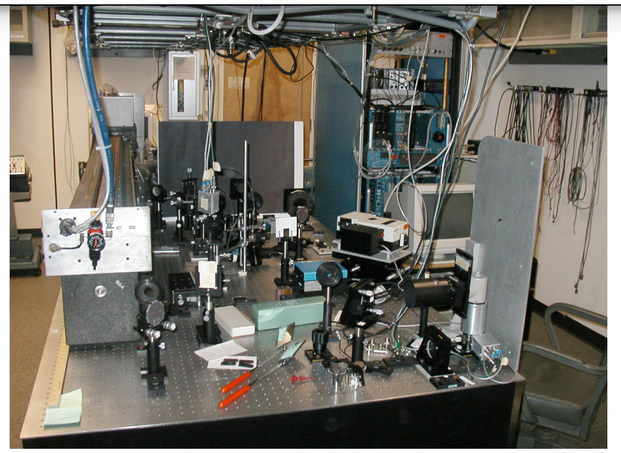
Decide on the **rejection r**... ...se total probability is small (**s**...

Do the experiment, collect data, compute the test statistic.

Make a **decision**: reject null hypothesis if the test statistic is in the rejection region.

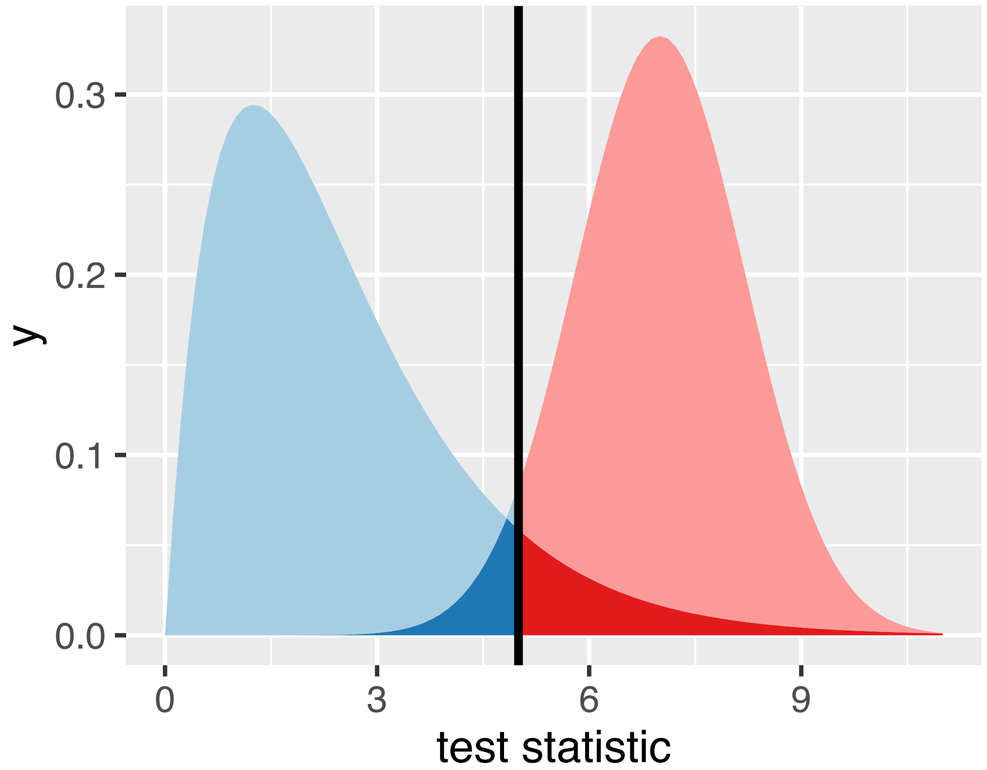
This is the idealised scenario, "orthodoxy".

Reality, esp. in retrospective 'data-mining' can be quite different.



Types of Error in Testing

Test vs reality	**Null hypothesis is true**	**. . . is false**
Reject null hypothesis	Type I error (false positive)	True positive
Do not reject	True negative	Type II error (false negative)



Parametric Theory vs Simulation

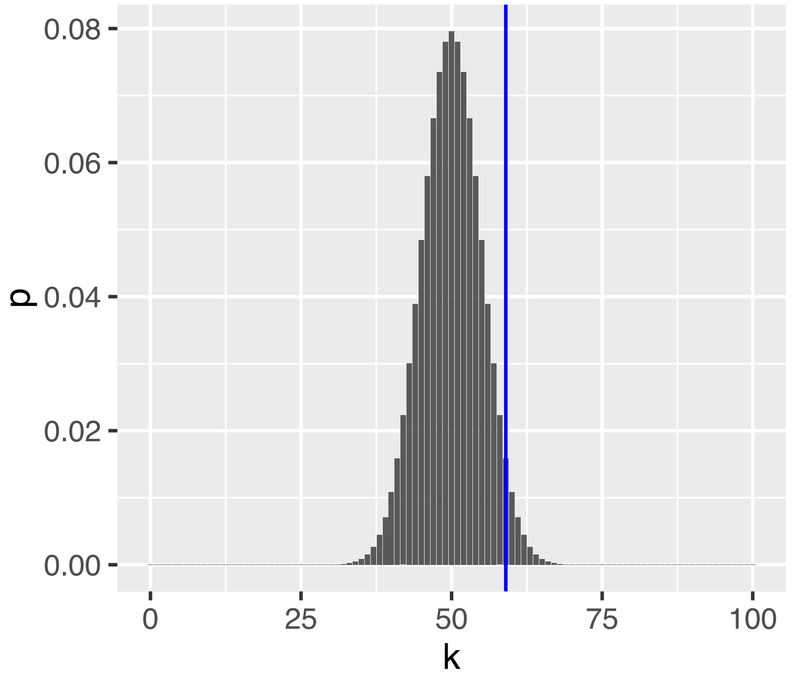


Figure 6.3: The binomial distribution for the parameters $n = 100$ and $p = 0.5$, according to Equation (6.1).

$$P(K = k \mid n, p) = \binom{n}{k} p^k \, (1-p)^{n-k}$$

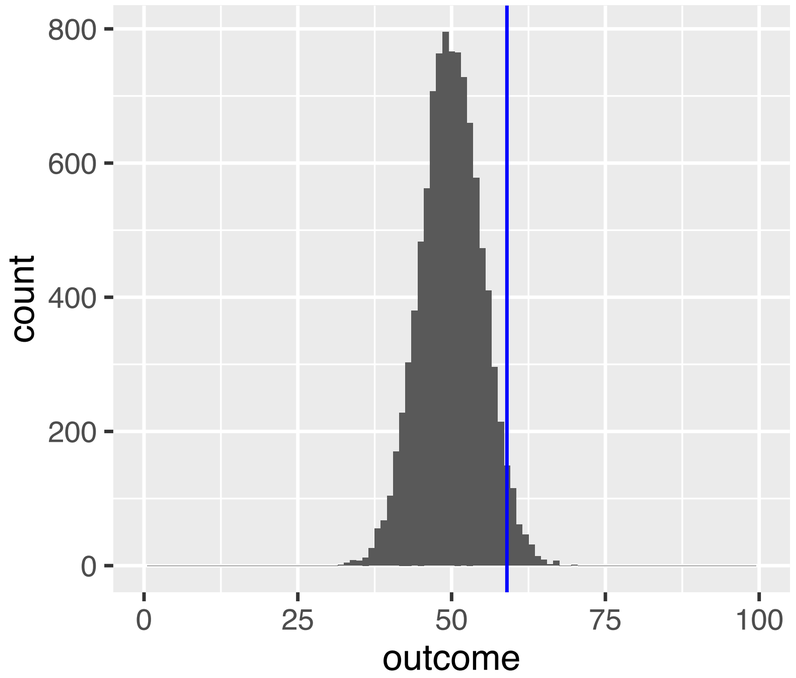
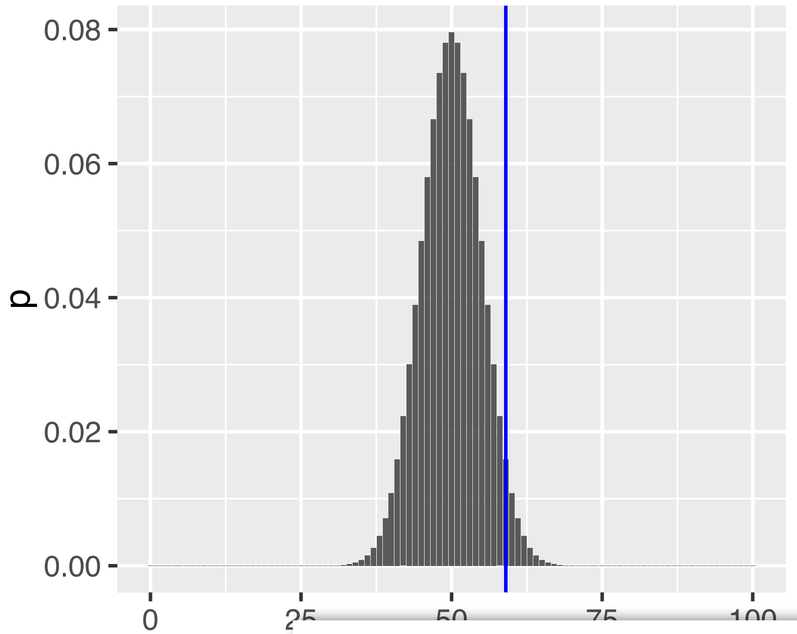


Figure 6.4: An approximation of the binomial distribution from 10^4 simulations (same parameters as Figure 6.3).

Parametric Theory vs Simulation



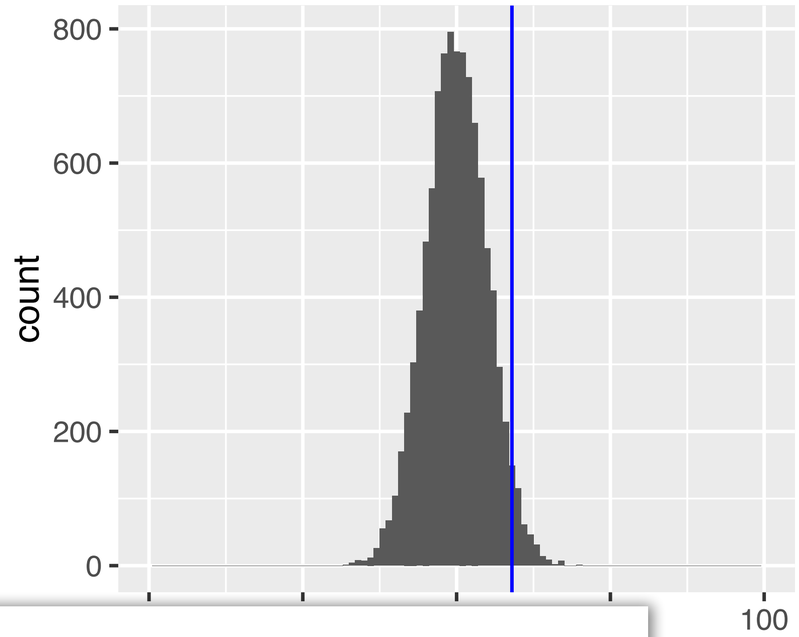
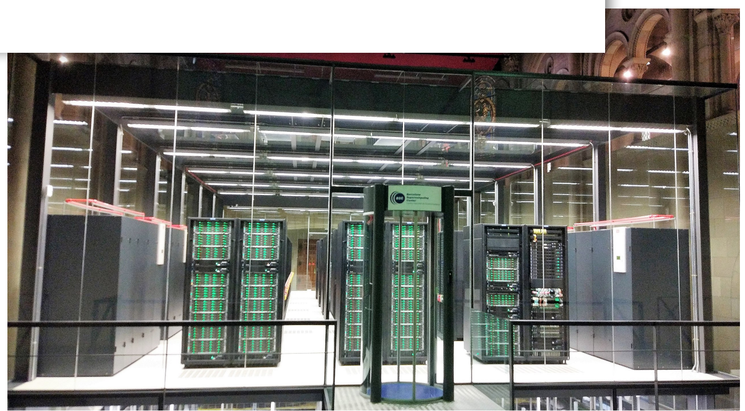


Figure 6.3: The b … the parameters n … according to Equ … ne … ulations

Q:
Discuss pros and contras for each

$$P(K = k \mid n, p) = \binom{n}{k} p^k \, (1-p)^{n-k}$$



The choice of the test statistic

Suppose we observed 50 tails in a row, and then 50 heads in a row. Is this a perfectly fair coin?

We could use a different test statistic: number of times we see two tails in a row

Is this statistic generally and always preferable?

Power

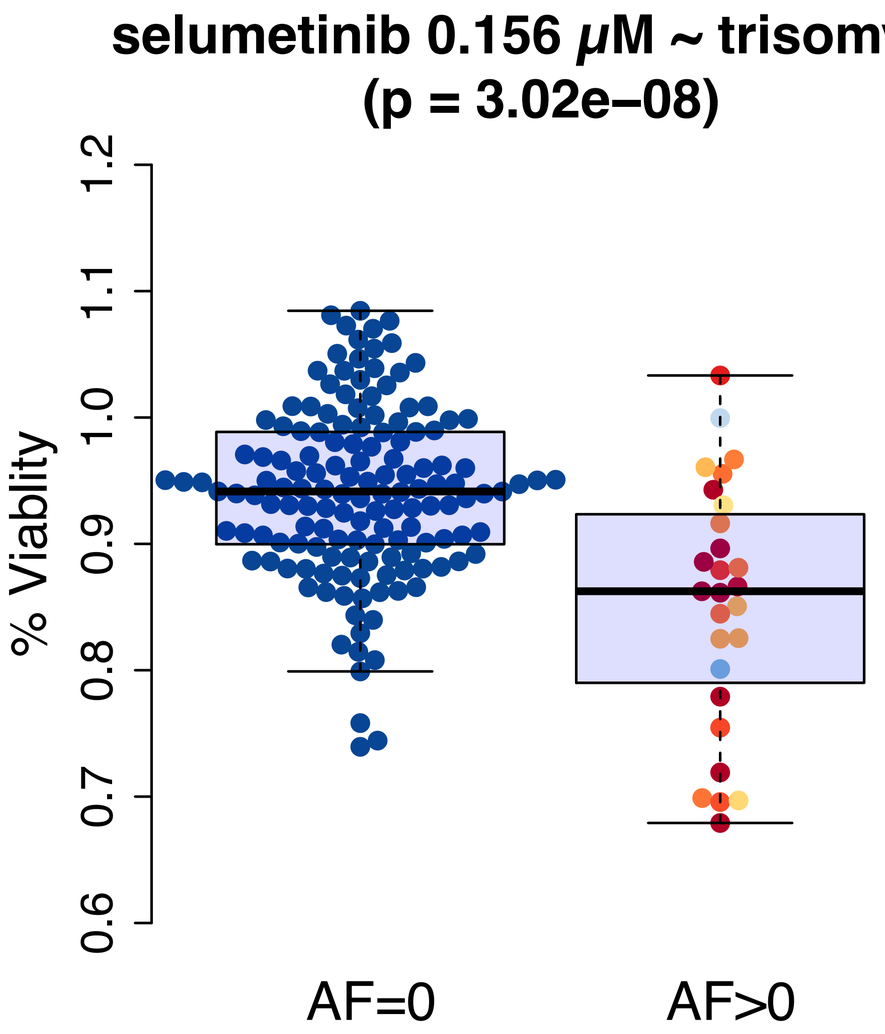
There can be several test statistics, with different power, for different types of alternative

Continuous data: the t-statistic

$$t = c\,\frac{m_1 - m_2}{s}$$

- Can also be adapted to one group only
- Relation to z-score

$$\begin{aligned} m_g &= \frac{1}{n_g}\sum_{i=1}^{n_g} x_{g,i} \qquad g = 1,2 \\ s^2 &= \frac{1}{n_1 + n_2 - 2}\left(\sum_{i=1}^{n_1}(x_{1,i} - m_1)^2 + \sum_{j=1}^{n_2}(x_{2,j} - m_2)^2\right) \\ c &= \sqrt{\frac{n_1 n_2}{n_1 + n_2}}. \end{aligned}$$



t-distribution

If the data are identically normal distributed and independent, then under H_0, t follows a 't-distribution' with parameter n_1+n_2 (a.k.a. degrees of freedom)

t-distribution

If the data are identically normal distributed and independent, then under H_0, t follows a 't-distribution' with parameter n_1+n_2 (a.k.a. degrees of freedom)

Q:
How does the distribution of |t| look?

t-distribution

If the data are identically normal distributed and independent, then under H_0, t follows a '*t*-distribution' with parameter n_1+n_2 (a.k.a. degrees of freedom)

Q:
How does the distribution of |t| look?

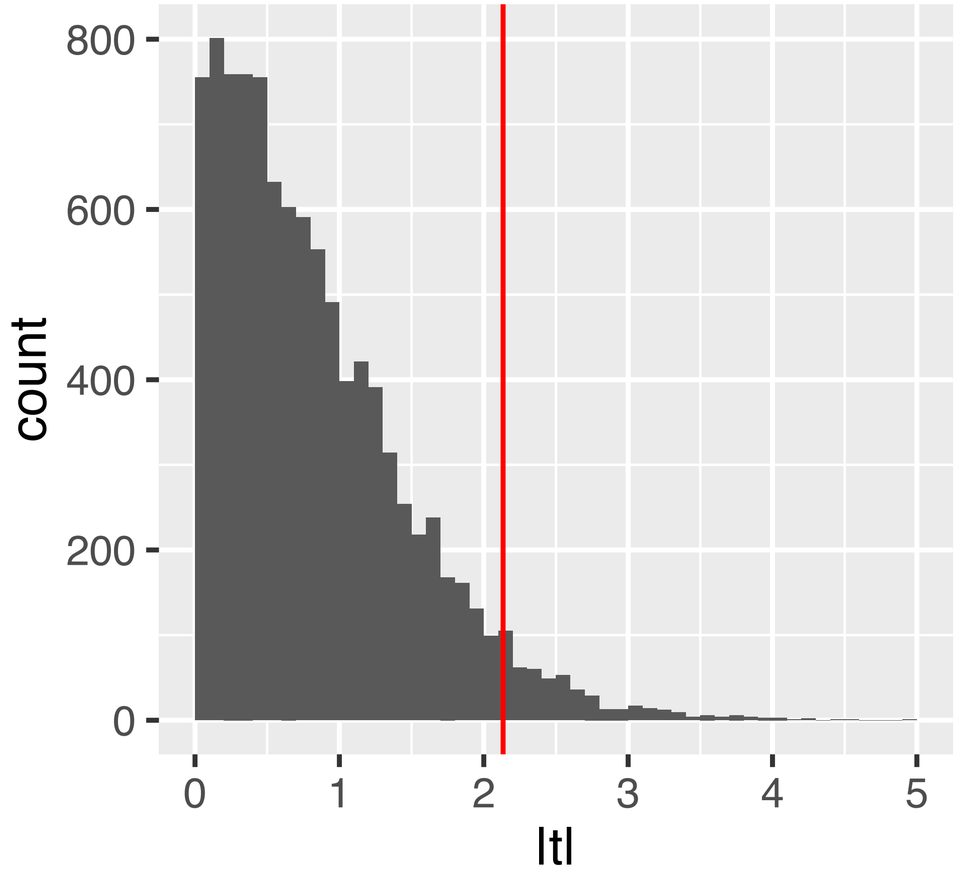


Figure 6.8: The null distribution of the (absolute) *t*-statistic determined by simulations – namely, by random permutations of the group labels.

Comments and Pitfalls

The proof that the *t*-statistic follows a *t*-distribution assumes that observations are independent and follow a normal distribution: this is a sufficient, but not necessary, condition

Comments and Pitfalls

The proof that the *t*-statistic follows a *t*-distribution assumes that observations are independent and follow a normal distribution: this is a sufficient, but not necessary, condition

Deviation from normality (heavier tails): test typically maintains type-I error control, but no longer has provably optimal power.

Comments and Pitfalls

The proof that the *t*-statistic follows a *t*-distribution assumes that observations are independent and follow a normal distribution: this is a sufficient, but not necessary, condition

Deviation from normality (heavier tails): test typically maintains type-I error control, but no longer has provably optimal power.
Options: use permutations;
use a different test (e.g., Wilcoxon)

Comments and Pitfalls

The proof that the *t*-statistic follows a *t*-distribution assumes that observations are independent and follow a normal distribution: this is a sufficient, but not necessary, condition

Deviation from normality (heavier tails): test typically maintains type-I error control, but no longer has provably optimal power.
Options: use permutations;
use a different test (e.g., Wilcoxon)

Deviation from independence: type-I error control is lost, p-values will likely be totally wrong (e.g., for positive correlation, too optimistic).

Comments and Pitfalls

The proof that the *t*-statistic follows a *t*-distribution assumes that observations are independent and follow a normal distribution: this is a sufficient, but not necessary, condition

Deviation from normality (heavier tails): test typically maintains type-I error control, but no longer has provably optimal power.
Options: use permutations;
use a different test (e.g., Wilcoxon)

Deviation from independence: type-I error control is lost, p-values will likely be totally wrong (e.g., for positive correlation, too optimistic).
No easy options:
… try to model the dependence / remove it …
… empirical null (Efron et al.) …

Avoid Fallacy

The p-value is the probability that the data could happen, under the condition that the null hypothesis is true.

It is not the probability that the null hypothesis is true.

Absence of evidence ≠ evidence of absence

Limitations of p-value based hypothesis testing

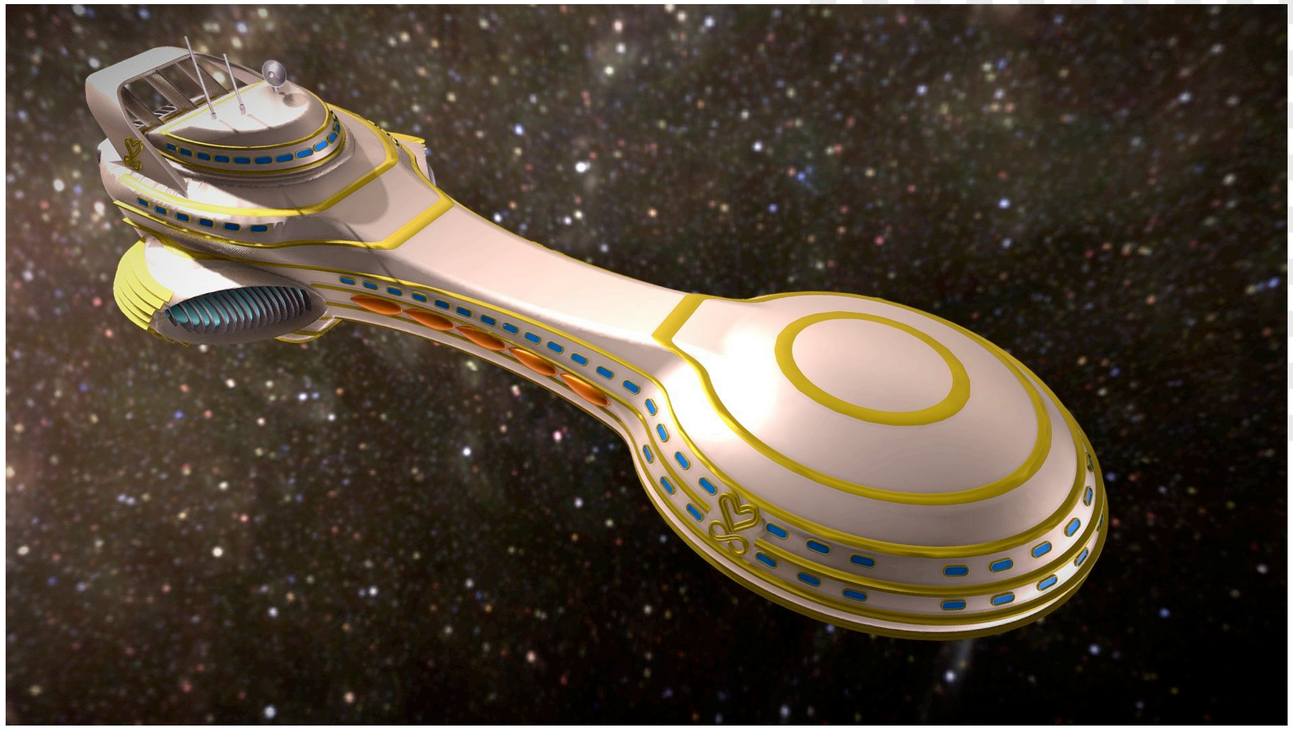
Too much power: often, the 'null' is small (point-like), alternative is large (region-like)…

…underlying / hidden confounders can create bogus rejections

Summarizing the data into one single number mushes together effect size and sample size

No place to take into account plausibility or 'prior' knowledge

Don’t report absurdly small p-values



Reporting p values, W. Huber, Cell Systems, DOI: 10.1016/j.cels.2019.03.001

What is p-value hacking ?

On the same data, try different tests until one is significant

On the same data, try different hypotheses until one is significant (HARKing - hypothesizing after results are known)

Moreover…:

retrospective data picking
'outlier' removal
the 5% threshold and publication bias

What is p-value hacking ?

On the same data, try different tests until one is significant

On the same data, try different hypotheses until one is significant (HARKing - hypothesizing after results are known)

Moreover…:
retrospective data picking
'outlier' removal
the 5% threshold and publication bias

The ASA's Statement on p-Values: Context, Process, and Purpose
Ronald L. Wasserstein & Nicole A. Lazara
DOI: 10.1080/00031305.2016.1154108

What is p-value hacking ?

On the same data, try different tests until one is significant

On the same data, try different hypotheses until one is significant (HARKing - hypothesizing after results are known)

Moreover…:
retrospective data picking
'outlier' removal
the 5% threshold and publication bias

The ASA's Statement on p-Values: Context, Process, and Purpose
Ronald L. Wasserstein & Nicole A. Lazara
DOI: 10.1080/00031305.2016.1154108

What can we do about this?

The right answer to the wrong question

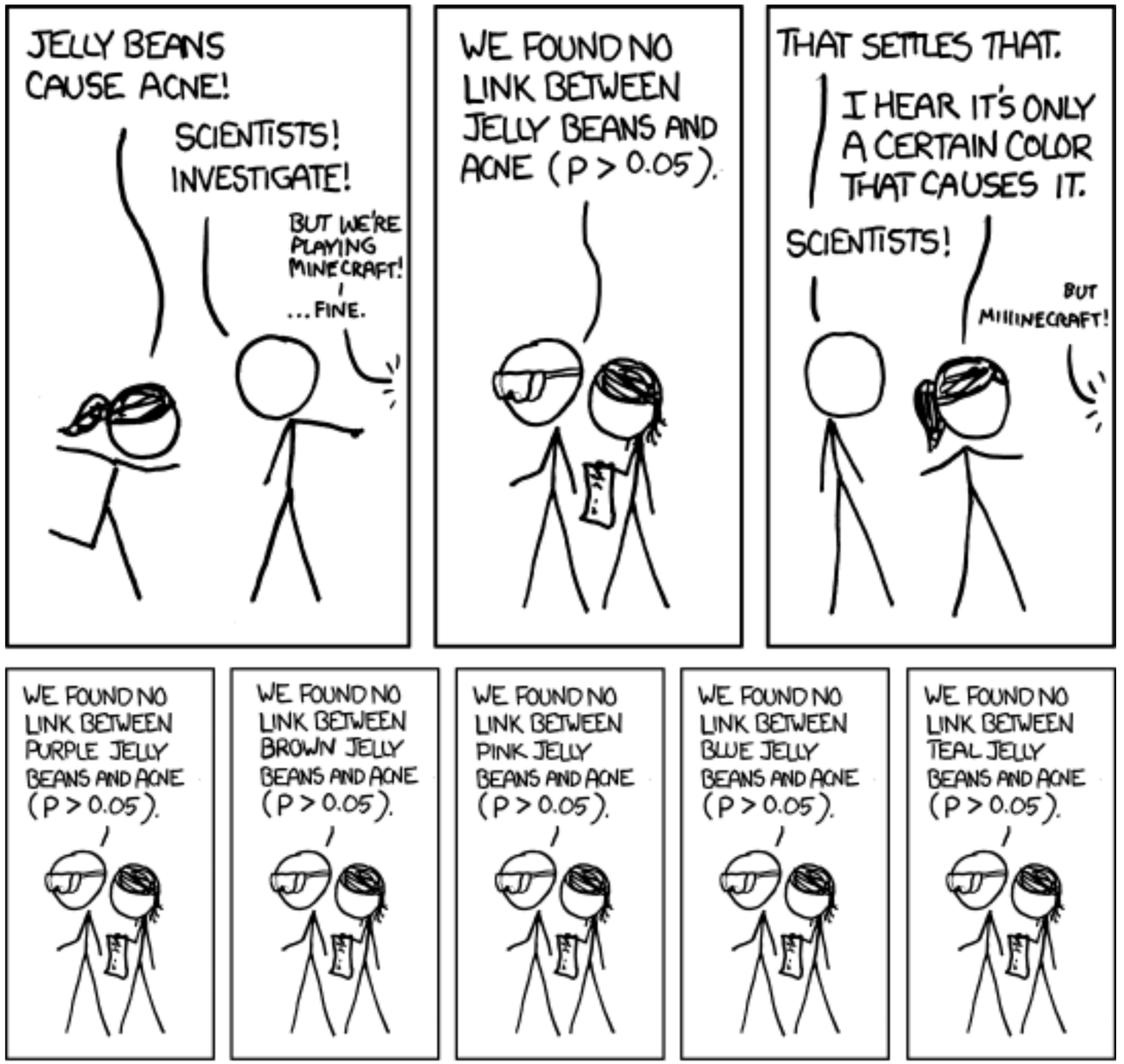
Researchers (regulators, investors, etc.) usually want to know:

If I publish this finding (allow this drug, invest in this product, ...), what is the probability that I'll later be proven wrong (cause harm, lose my money, ...)?

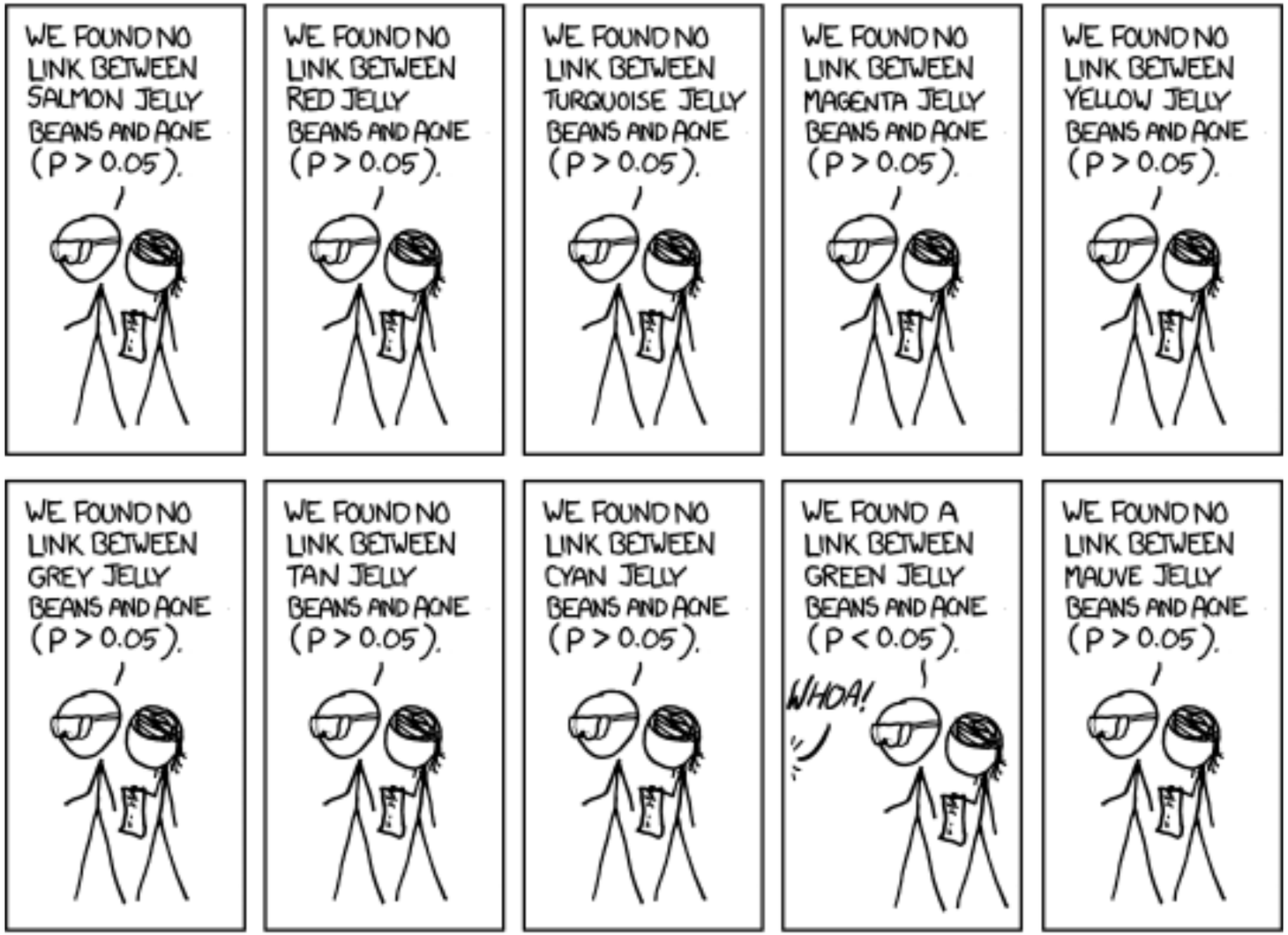
The p-value is the probability of seeing the data if the null hypothesis is true. It has little to do with the probability that my subsequent decision is wrong (a.k.a. "false discovery").

Can we compute a *false discovery probability* instead?

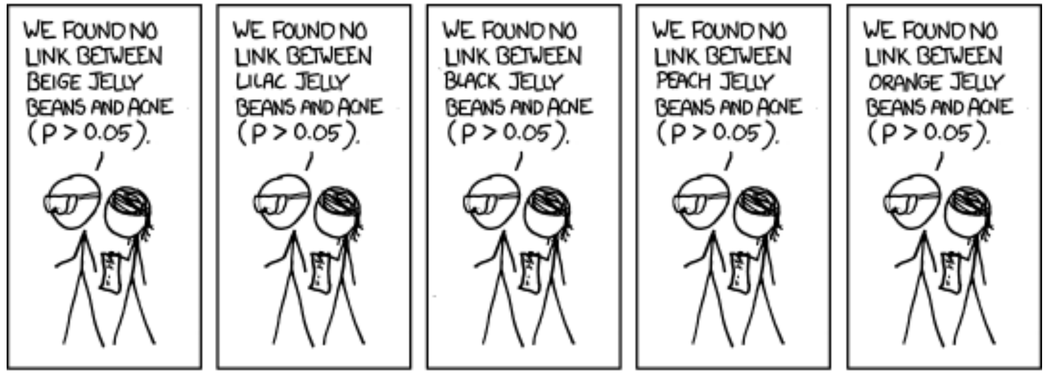
Multiple Testing

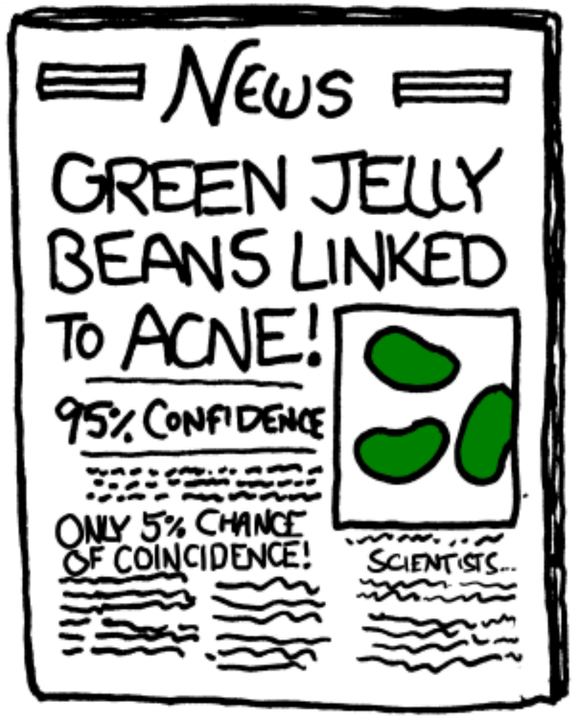


Multiple Testing



Multiple Testing

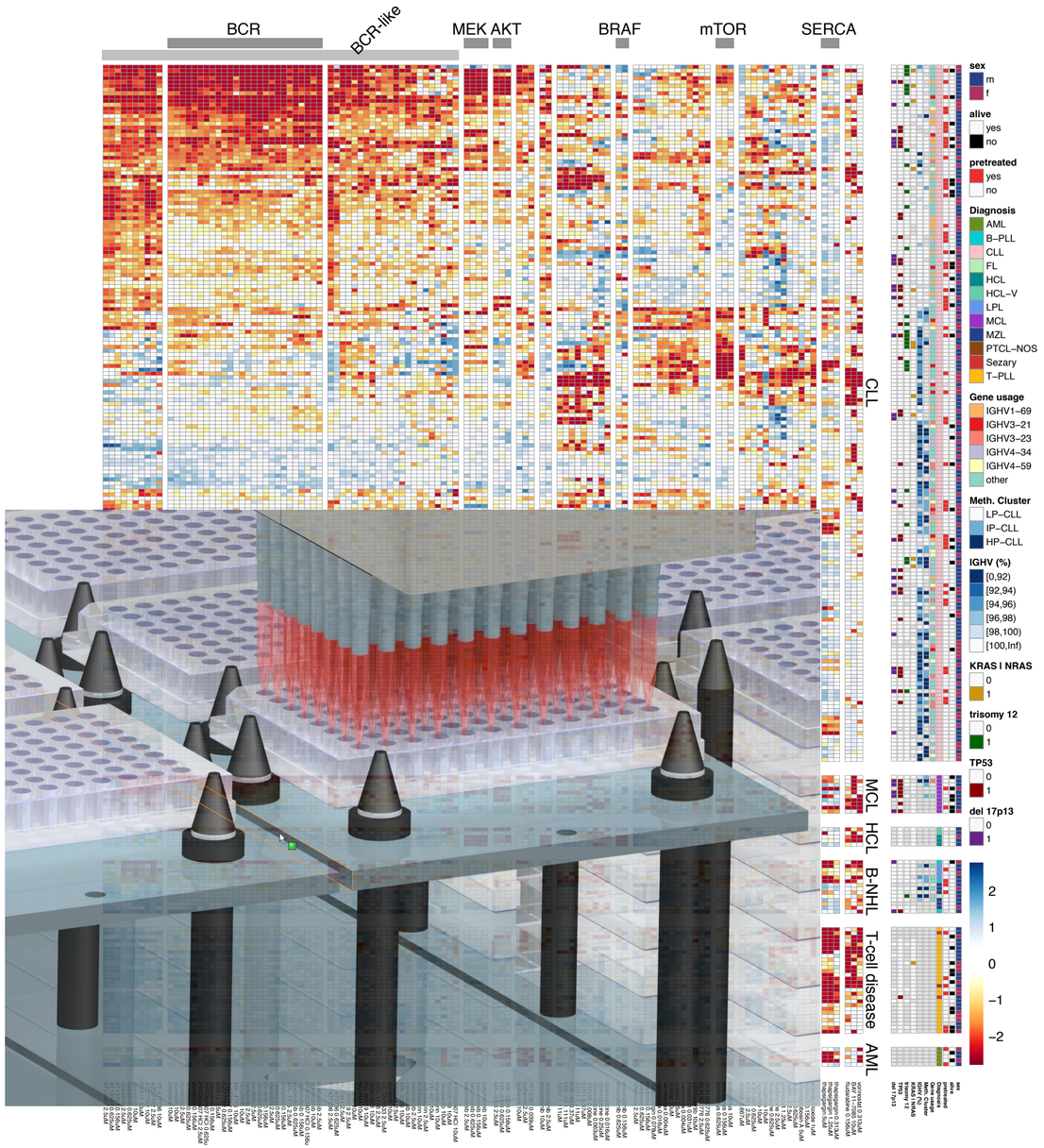




Multiple Testing

Many data analysis approaches in genomics employ item-by-item testing:

- Expression profiling
- Differential microbiome analysis
- Genetic or chemical compound screens
- Genome-wide association studies
- Proteomics
- Variant calling
- …



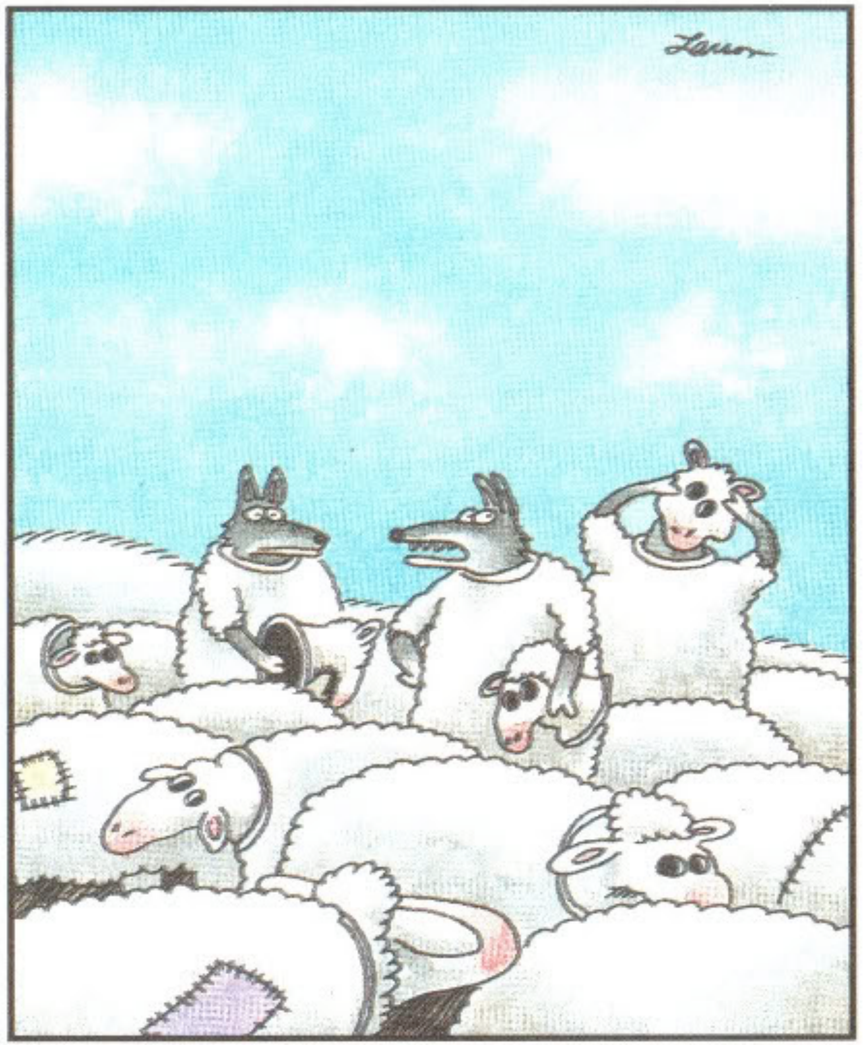
False Positive Rate and False Discovery Rate

FPR: fraction of FP among all true negatives

FDR: fraction of FP among hits called

Example:
20,000 genes, 500 are d.e.,100 hits called, 10 of them wrong.

FPR: 10/19,500 ≈ 0.05%
FDR: 10/100 = 10%



"Wait a minute! Isn't anyone here a real sheep?"

The Multiple Testing Burden

When performing several tests, type I error goes up: for $\alpha = 0.05$ and n indep. tests, probability of no false positive result is

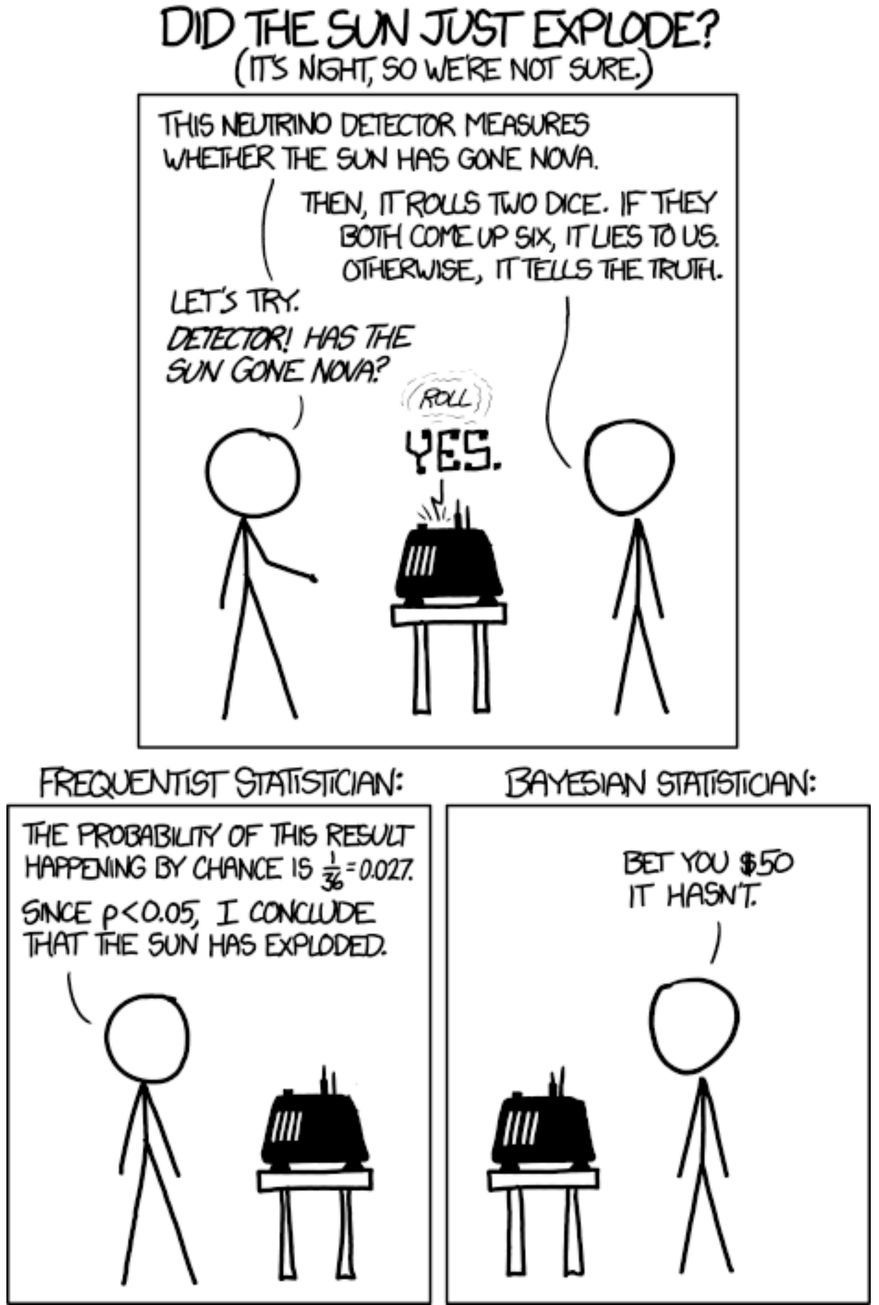
$$\underbrace{0.95 \cdot 0.95 \cdot \ldots \cdot 0.95}_{\text{n–times}} \lll 0.95$$

Bonferroni Correction

For *m* tests, multiply each *p*-value with *m*.

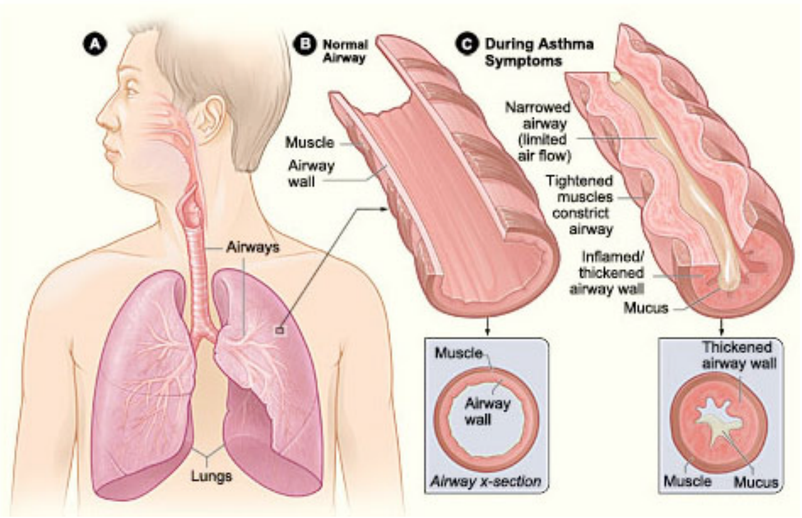
Then see if anyone still remains below *α*.

The Multiple Testing Opportunity



Example data set: RNA-Seq

Transcriptome changes in four samples of primary human airway smooth muscle cells treated with dexamethasone, a synthetic glucocorticoid. 1 µM for 18 h.



cellline	dexamethasone
N61311	untrt
N61311	trt
N052611	untrt
N052611	trt
N080611	untrt
N080611	trt
N061011	untrt
N061011	trt

DESeq2 differential expression analysis:

gene i, sample j:

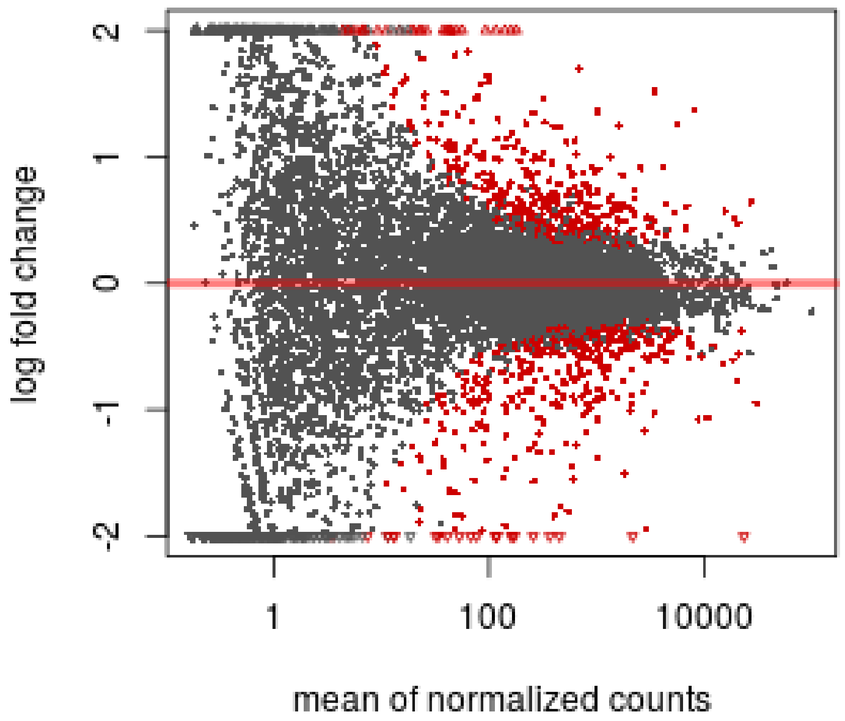
$$K_{ij} \sim \text{NB}(\text{mean} = \mu_{ij}, \text{dispersion} = \alpha_j)$$
$$\mu_{ij} = s_j q_{ij}$$
$$\log q_{ij} = \sum_r x_{jr}\beta_{rj}$$

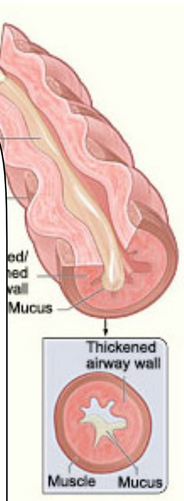
```
design <-  ~ cellline + dexamethasone
```

Himes et al. "RNA-Seq Transcriptome Profiling Identifies CRISPLD2 as a Glucocorticoid Responsive Gene that Modulates Cytokine Function in Airway Smooth Muscle Cells." PLoS One. 2014 GEO: GSE52778.

Example data set: RNA-Seq

Transcrip
sample
smooth
dexame
glucocc





cell	
N61	
N61	
N05	
N05	
N08	
N08	
N0610	
N061011	trt

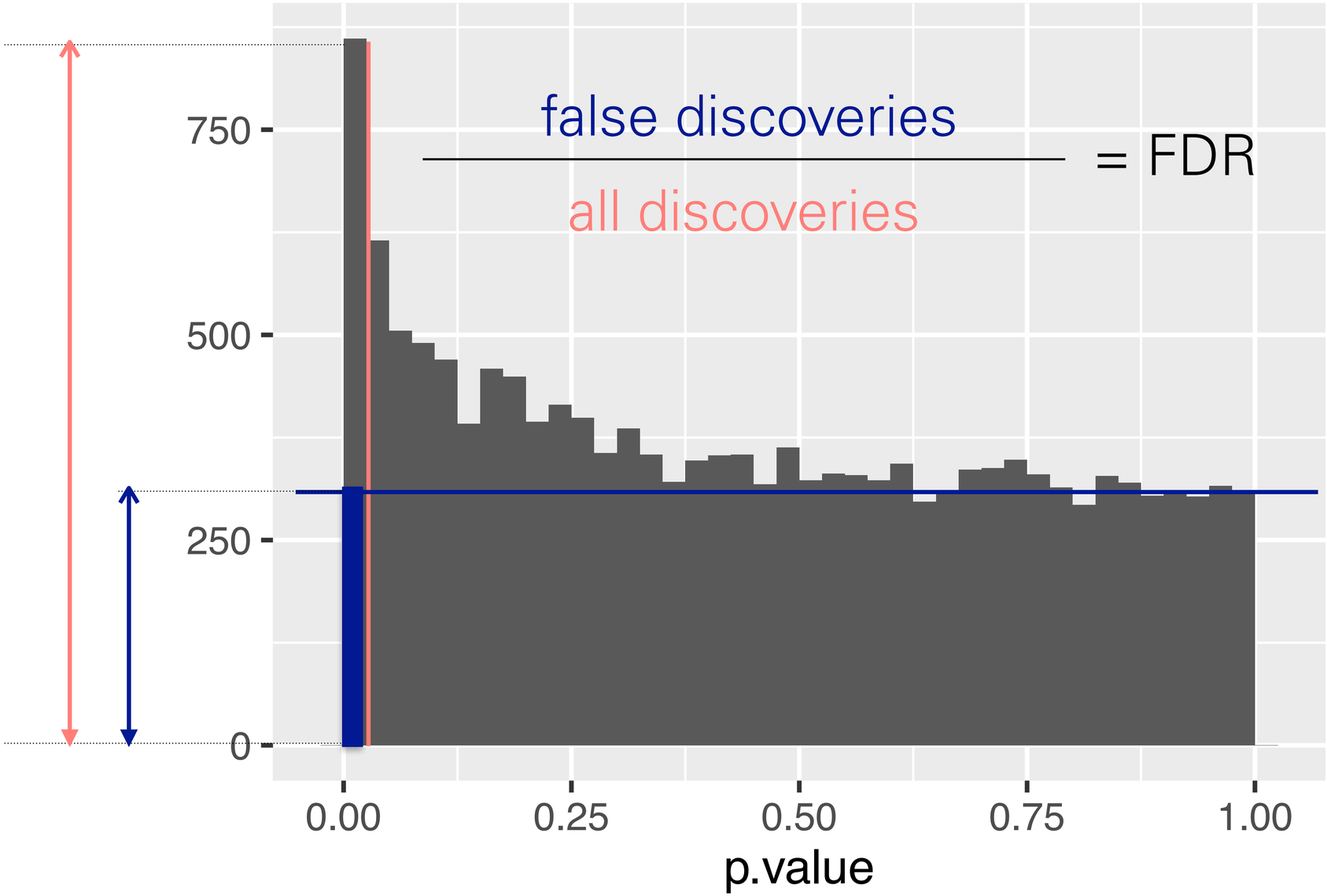
analysis:

persion $= \alpha_j$)

design <- ~ cellline + dexamethasone

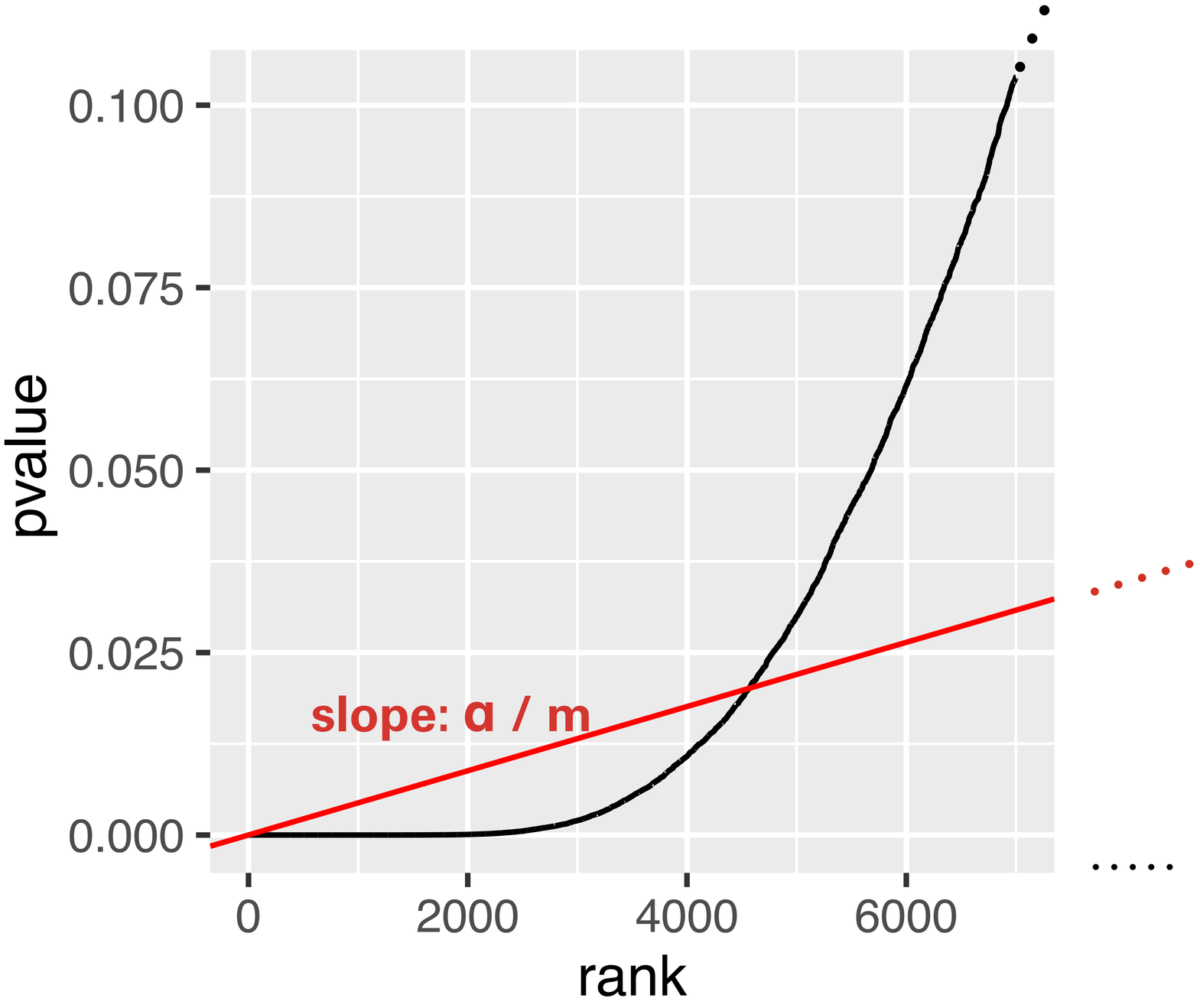
Himes et al. "RNA-Seq Transcriptome Profiling Identifies CRISPLD2 as a Glucocorticoid Responsive Gene that Modulates Cytokine Function in Airway Smooth Muscle Cells." PLoS One. 2014 GEO: GSE52778.

False Discovery Rate



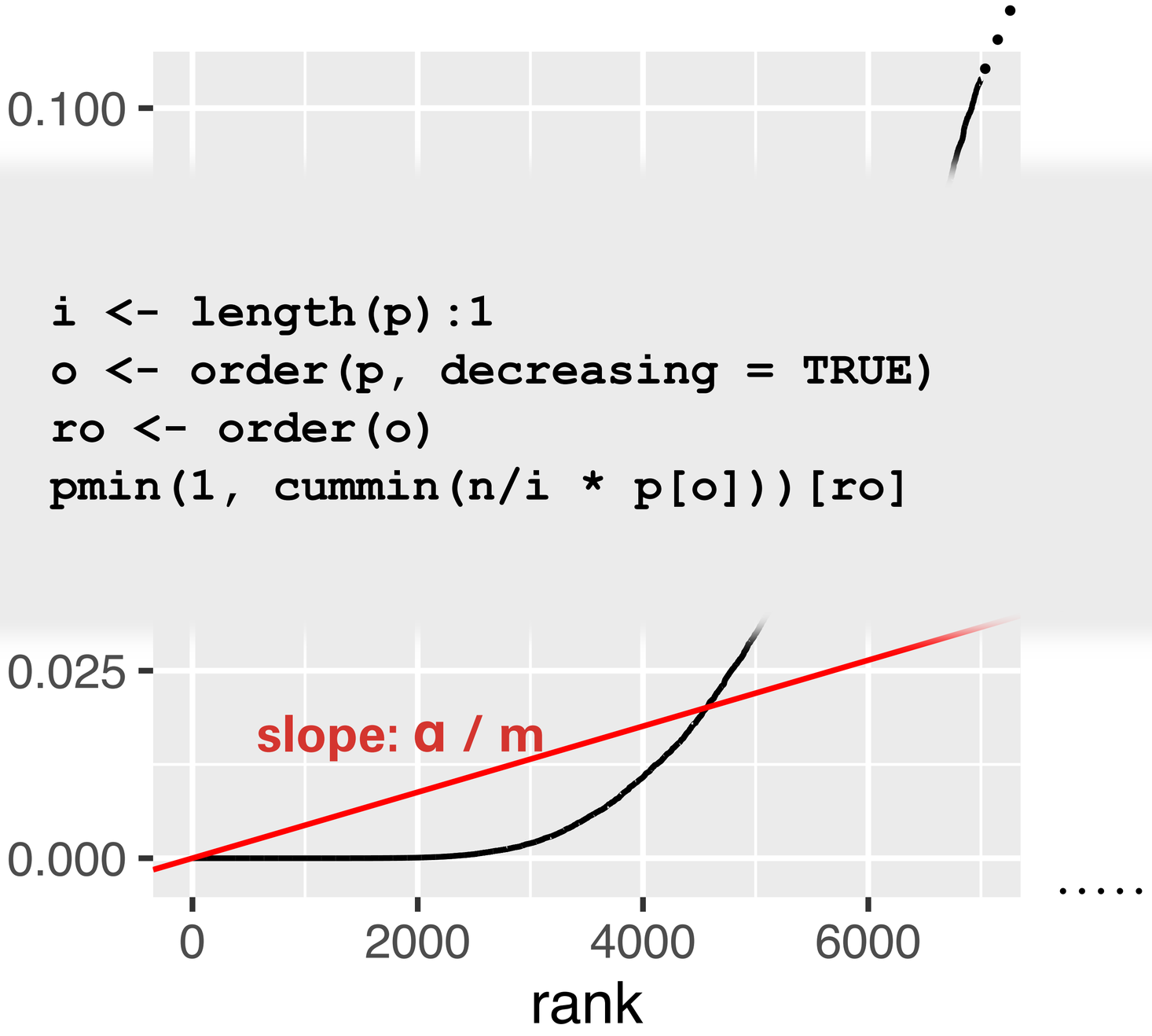
Method of Benjamini & Hochberg (1995)

Method of Benjamini & Hochberg



Method of Benjamini & Hochberg

```
BH = {
        i <- length(p):1
        o <- order(p, decreasing = TRUE)
        ro <- order(o)
        pmin(1, cummin(n/i * p[o]))[ro]
    }
```



Not all Hypothesis Tests are Created Equal

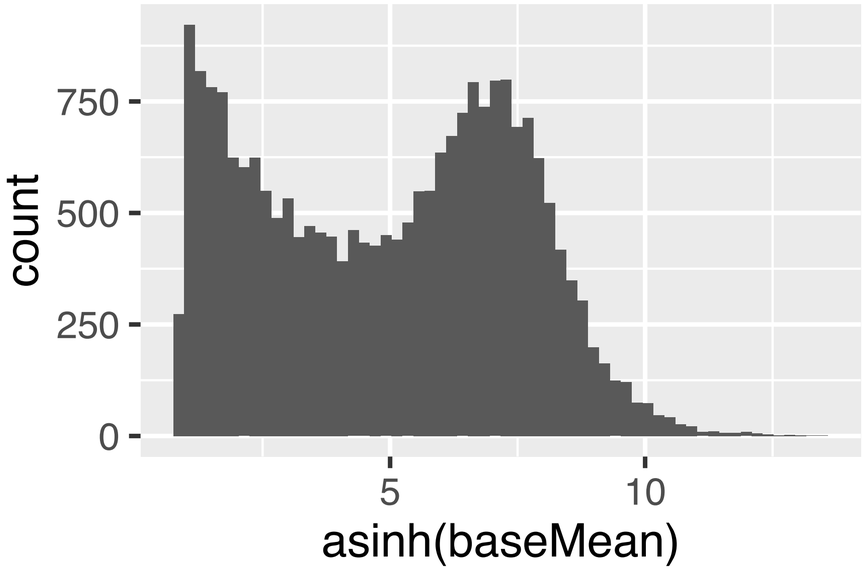
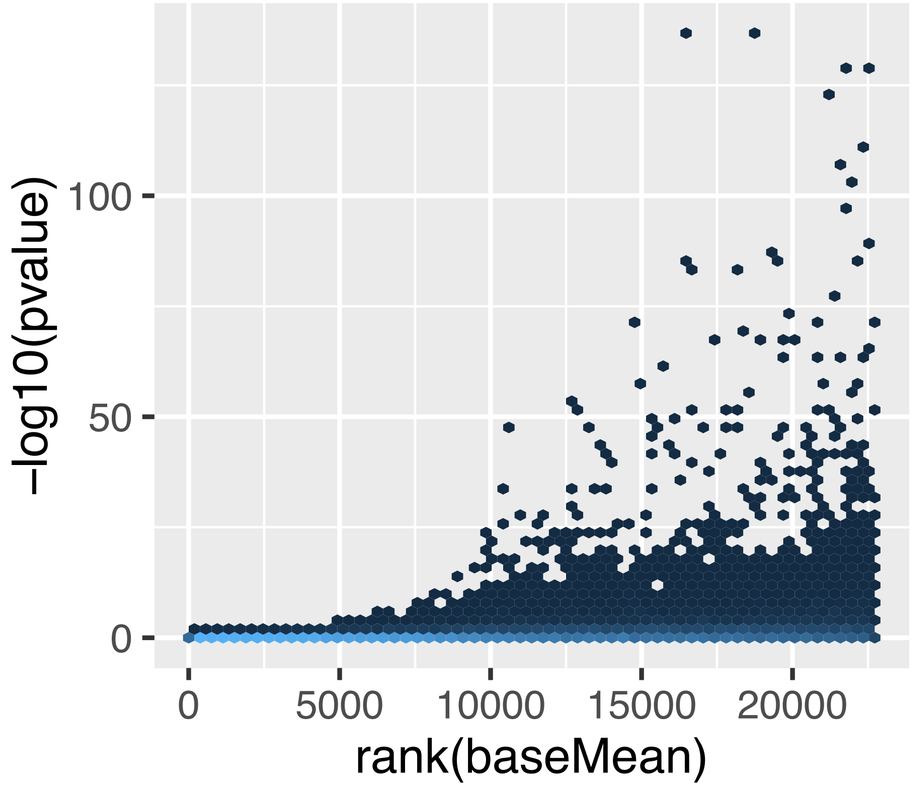


Figure 6.15: Histogram of `baseMean`. We see that it covers a large dynamic range, from close to 0 to around 3.3×10^5.



Covariates - examples

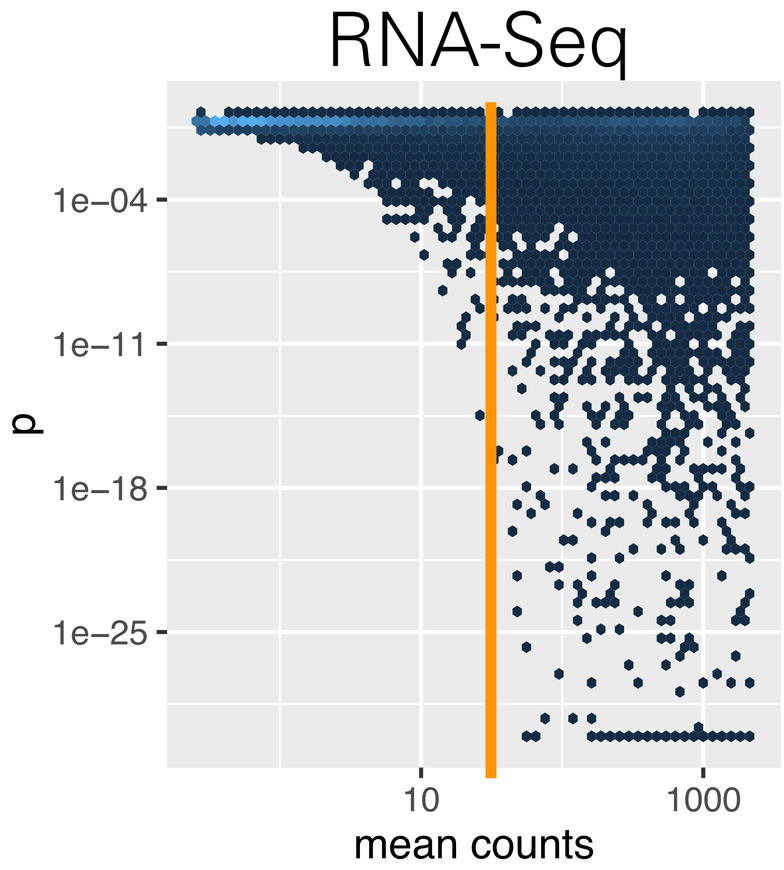
Application	Covariate
Differential RNA-Seq, ChIP-Seq, CLIP-seq, . . .	(Normalized) mean of counts for each gene
eQTL analysis	SNP – gene distance
GWAS	Minor allele frequency
t-tests	Overall variance
Two-sided tests	Sign
All applications	Sample size; measures of signal-to-noise ratio

Independent Filtering

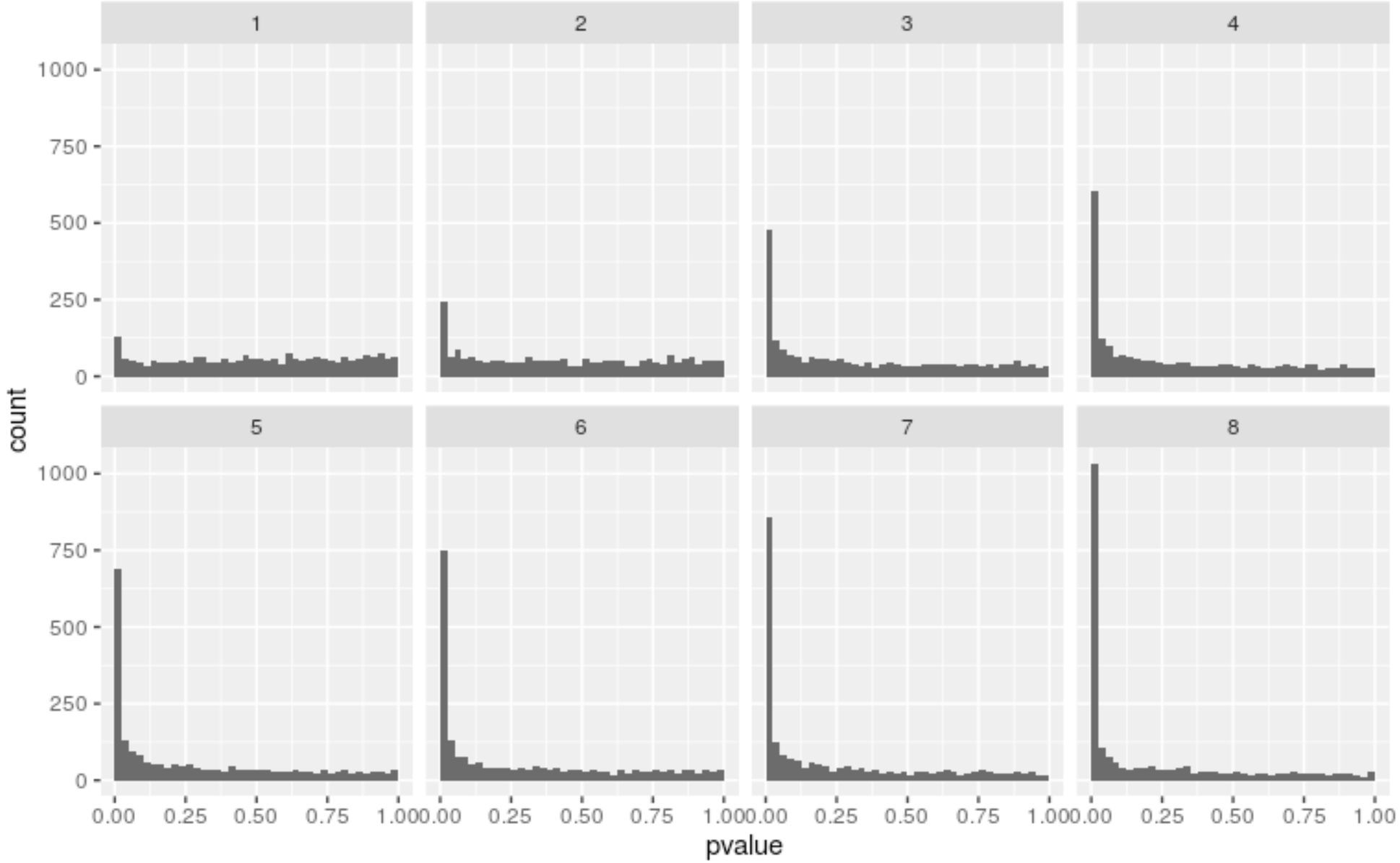
Two steps:

- All hypotheses H_i with $X_i < x$ get filtered.
- Apply BH to remaining hypotheses.

(Bourgon, Gentleman, Huber *PNAS* 2010)



RNA-Seq p-value histogram stratified by average read count



Weighted Benjamini-Hochberg method

- Let $w_i \geq 0$ and $\frac{1}{m}\sum_{i=1}^{m} w_i = 1$ ("weight budget").
- Define $Q_i = P_i / w_i$.
- Apply BH to Q_i instead of P_i.
- Proven Type-I error (FDR) control (Genovese, Roeder, Wasserman *Biometrika* 2006).
- If $w_i > 1$, then H_i is easier to reject.
- $Q_i \leq t \Leftrightarrow P_i \leq w_i t =: t_i$

Weighted Benjamini-Hochberg method

- Let $w_i \geq 0$ and $\frac{1}{m}\sum_{i=1}^{m} w_i = 1$ ("weight budget").
- Define $Q_i = P_i / w_i$.
- Apply BH to Q instead of P
- Proven Ty der, Wasserma
- If $w_i > 1$,
- $Q_i \leq t \Leftrightarrow$

Weighted Benjamini-Hoch[illegible]g method

- Let $w_i \geq 0$ and $\frac{1}{m}\sum_{i=1}^{m}$ [illegible] budget").
- Define $Q_i =$ [illegible]
- Apply [illegible]
- Proven [illegible] der, Wasserma[illegible]
- If $w_i > 1$, [illegible]
- $Q_i \leq t \Leftrightarrow$ [illegible]

Problem: how to know the weights?

Independent hypothesis weighting (IHW)

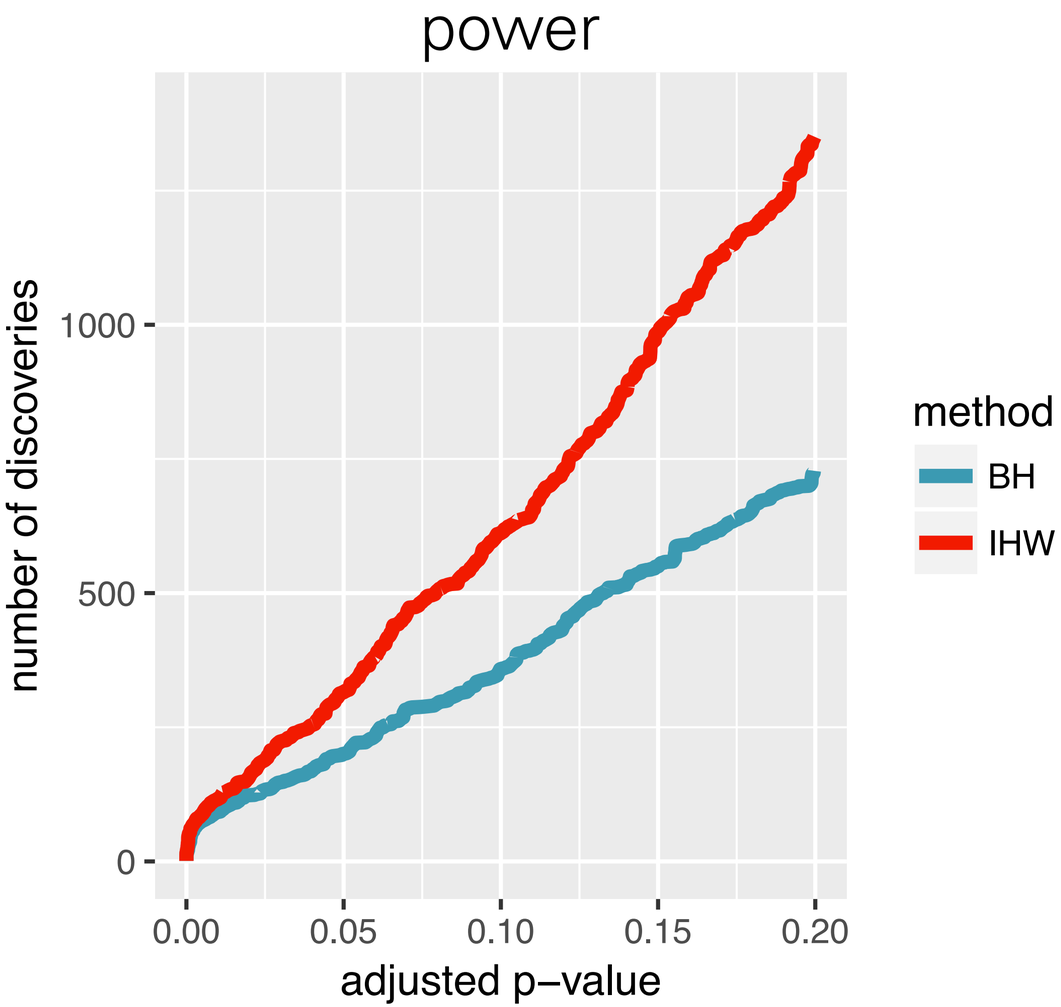
- Stratify the tests into G bins, by covariate X
- Choose α
- For each possible weight vector $\mathbf{w} = (w_1, \ldots, w_G)$ apply weighted BH procedure. Choose $\mathbf{w}$ that maximizes the number of rejections at level α.
- Report the result with the optimal weight vector $\mathbf{w}^*$.

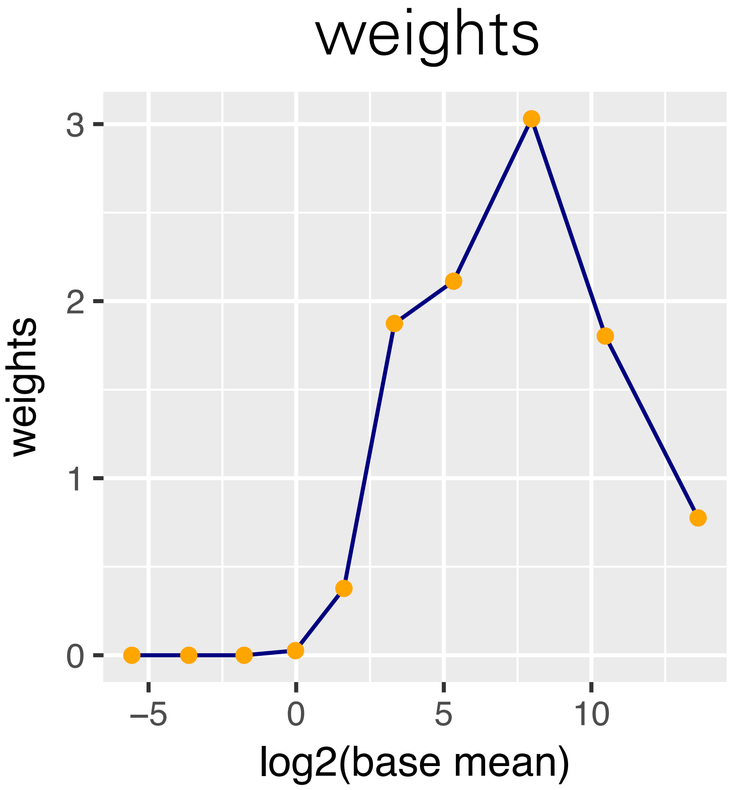
Nikos Ignatiadis

Ignatiadis et al.,
- Nature Methods 2016, DOI10.1038/nmeth.3885
- arXiv:1701.05179

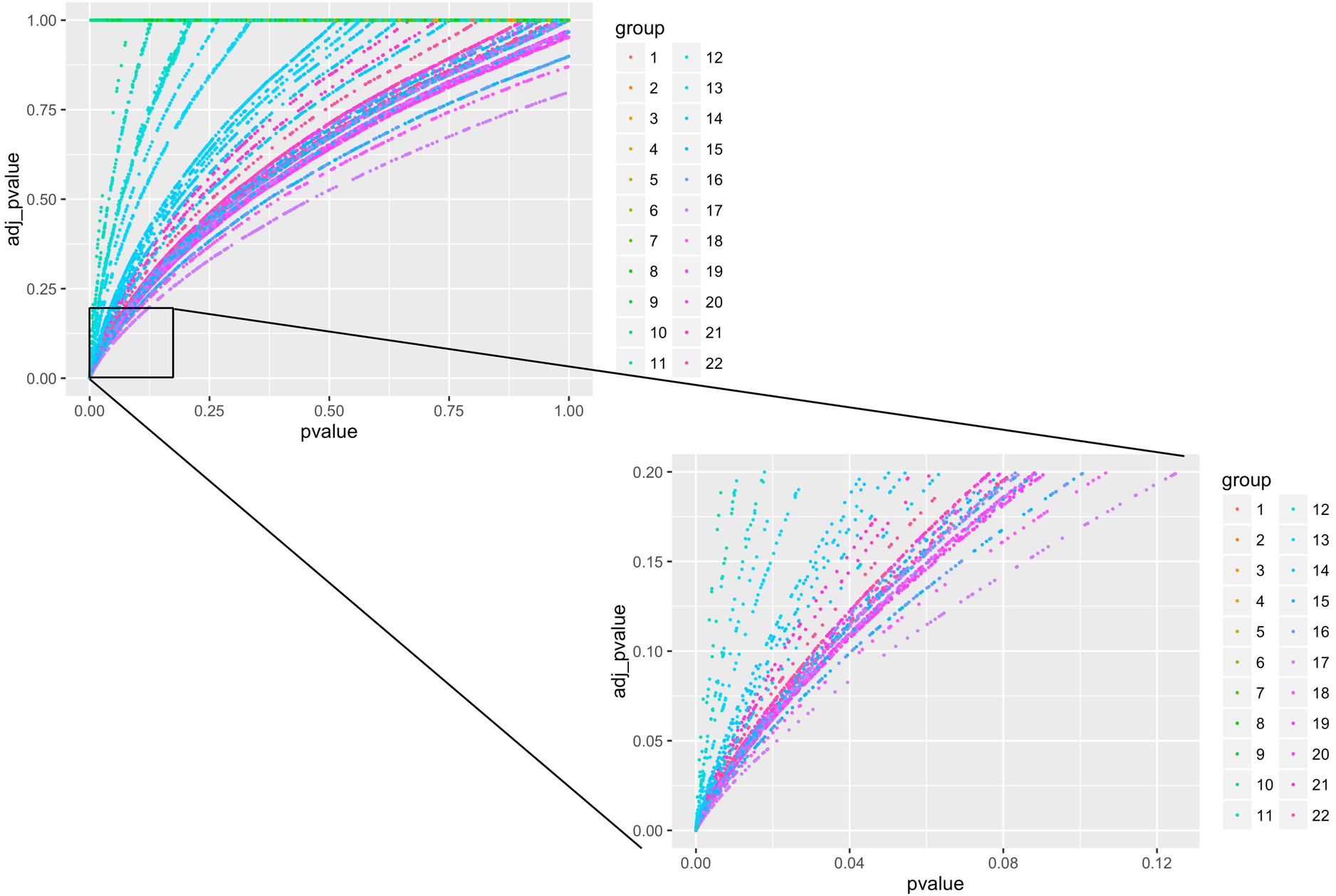
Bioconductor package IHW

RNA-Seq example (DESeq2)

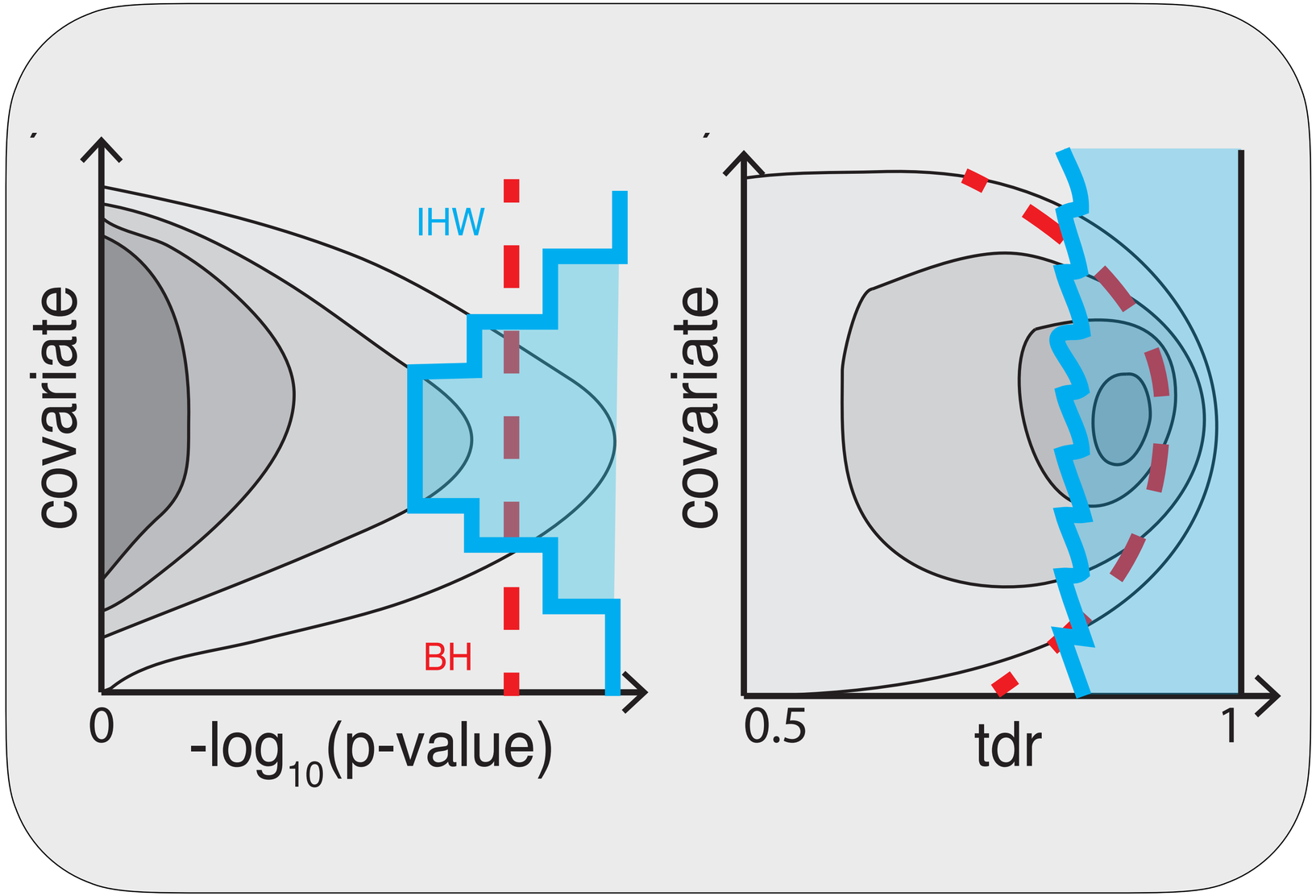




Ranking is not monotonous in raw p-values

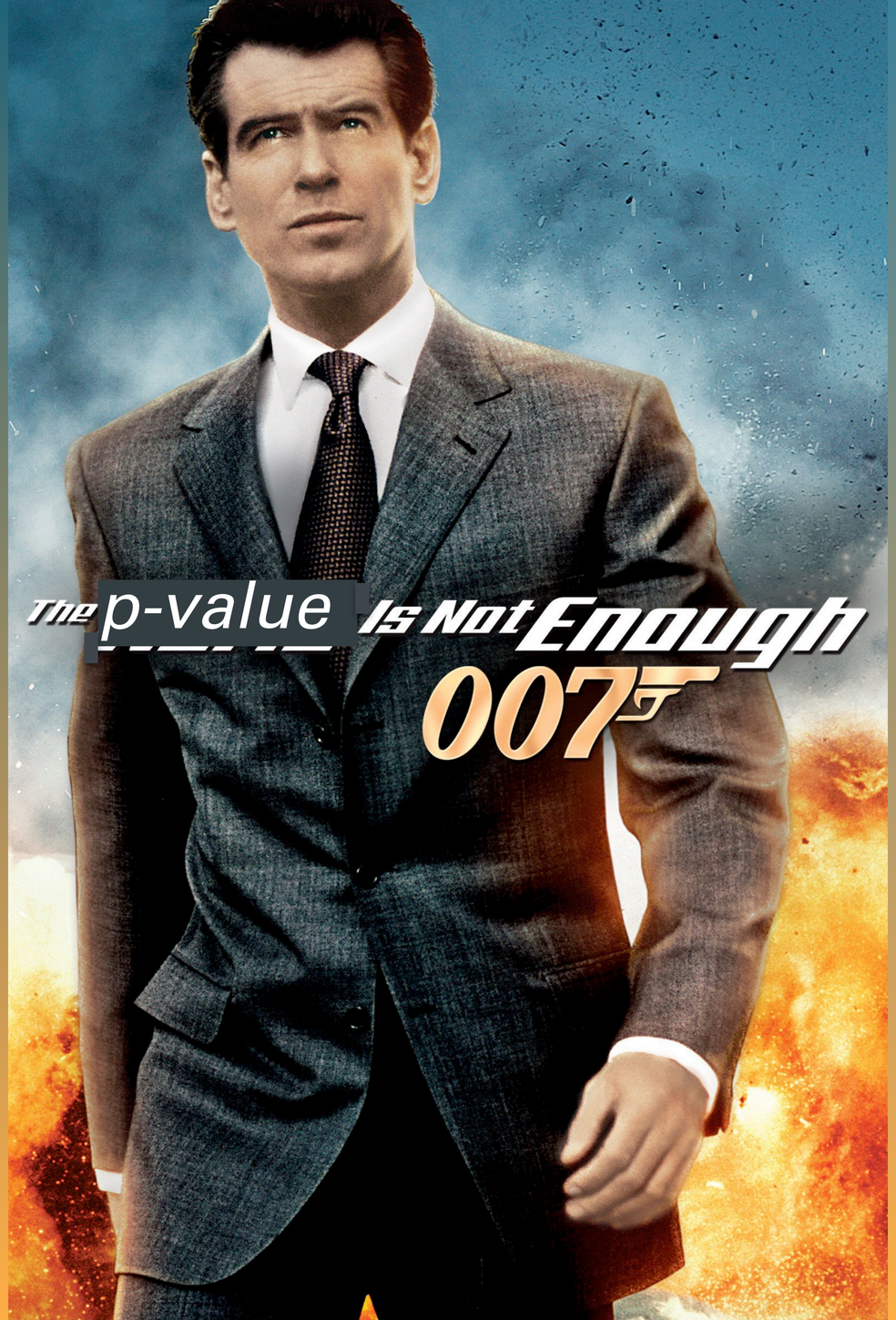


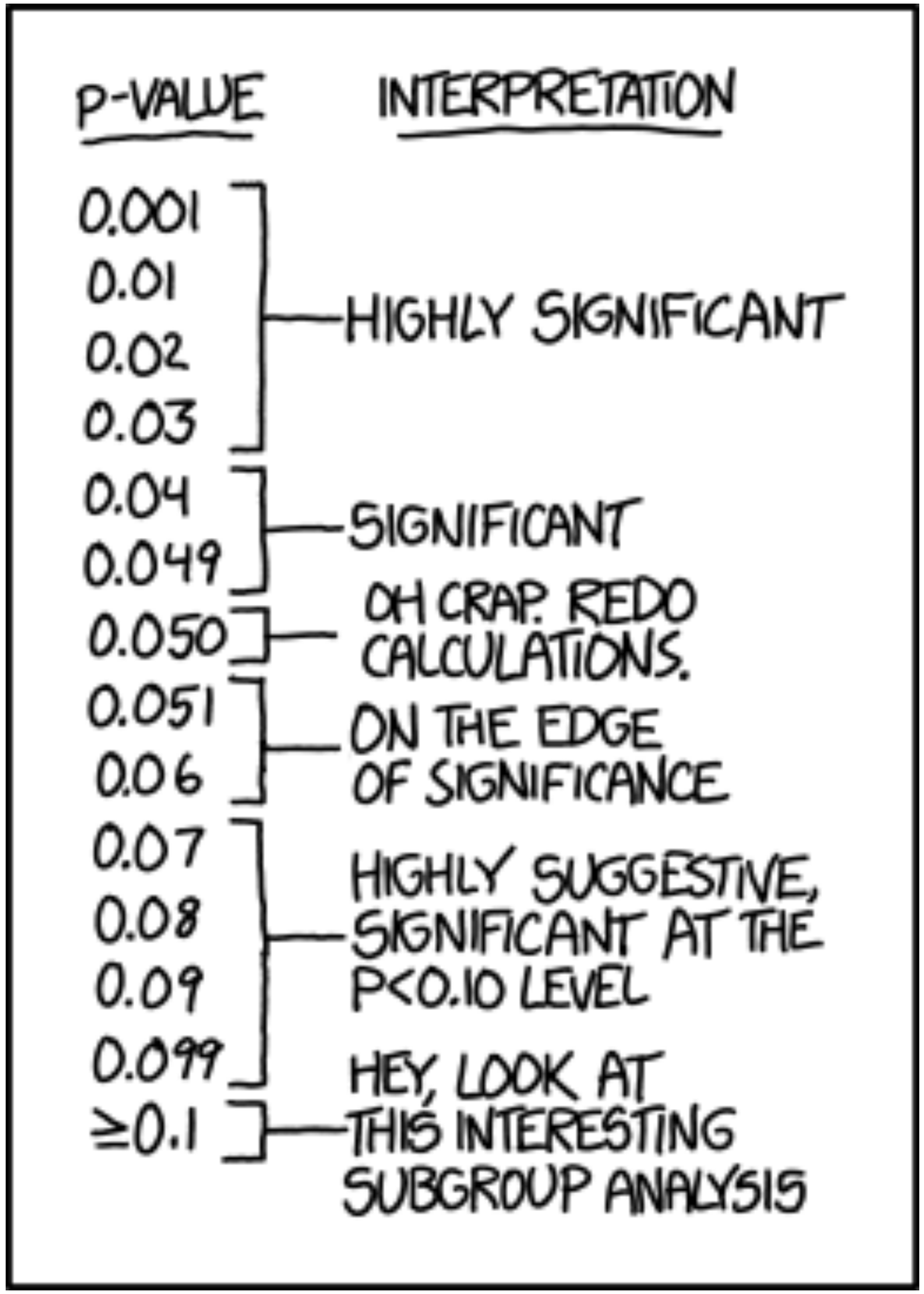
The decision boundaries is in two dimensions



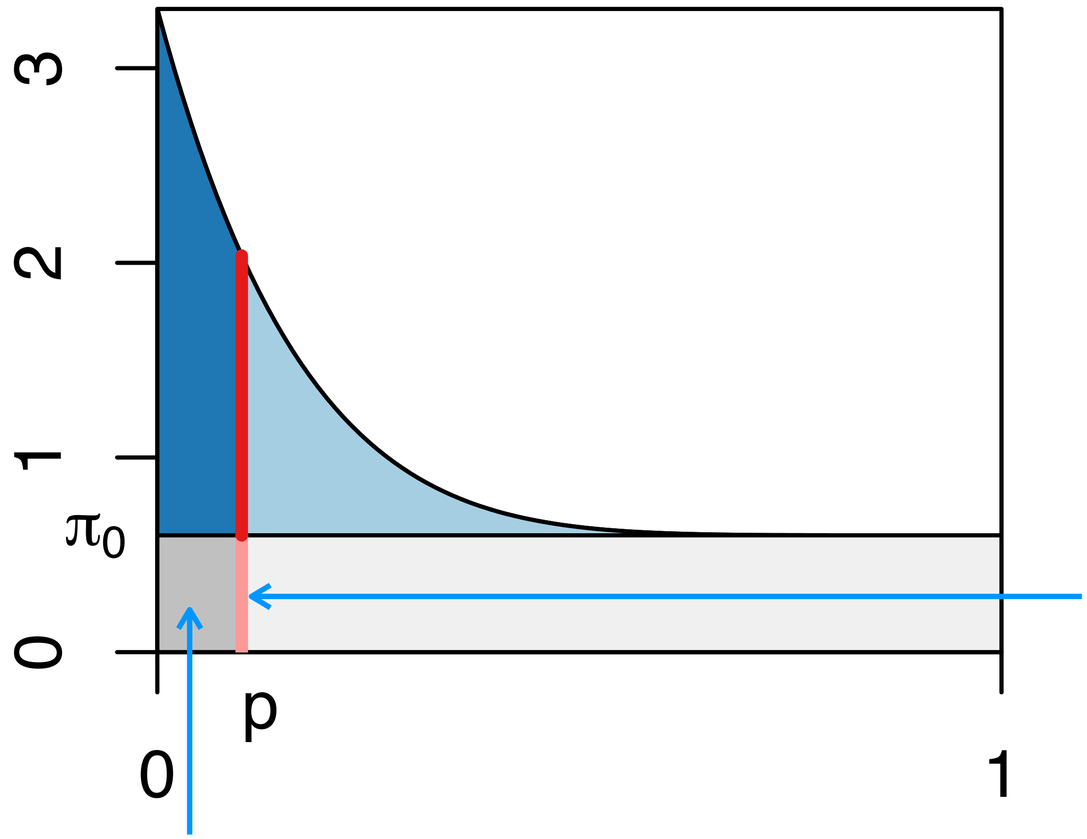
Summary

- Multiple testing is not a problem but an opportunity
- Heterogeneity across tests
- Informative covariates are often apparent to domain scientists
  - independent of test statistic under the null
  - informative on π_1, F_{alt}
- Can do data-driven weighting ("IHW")
  - Scales well to millions of hypotheses
  - Controls 'overoptimism'


The p-value Is Not Enough
007


P-VALUE
INTERPRETATION
0.001
0.01
0.02
0.03
HIGHLY SIGNIFICANT
0.04
0.049
SIGNIFICANT
0.050
OH CRAP. REDO CALCULATIONS.
0.051
0.06
ON THE EDGE OF SIGNIFICANCE
0.07
0.08
0.09
HIGHLY SUGGESTIVE, SIGNIFICANT AT THE P<0.10 LEVEL
0.099
≥0.1
HEY, LOOK AT THIS INTERESTING SUBGROUP ANALYSIS

The two-groups model and the (local) false discovery rate



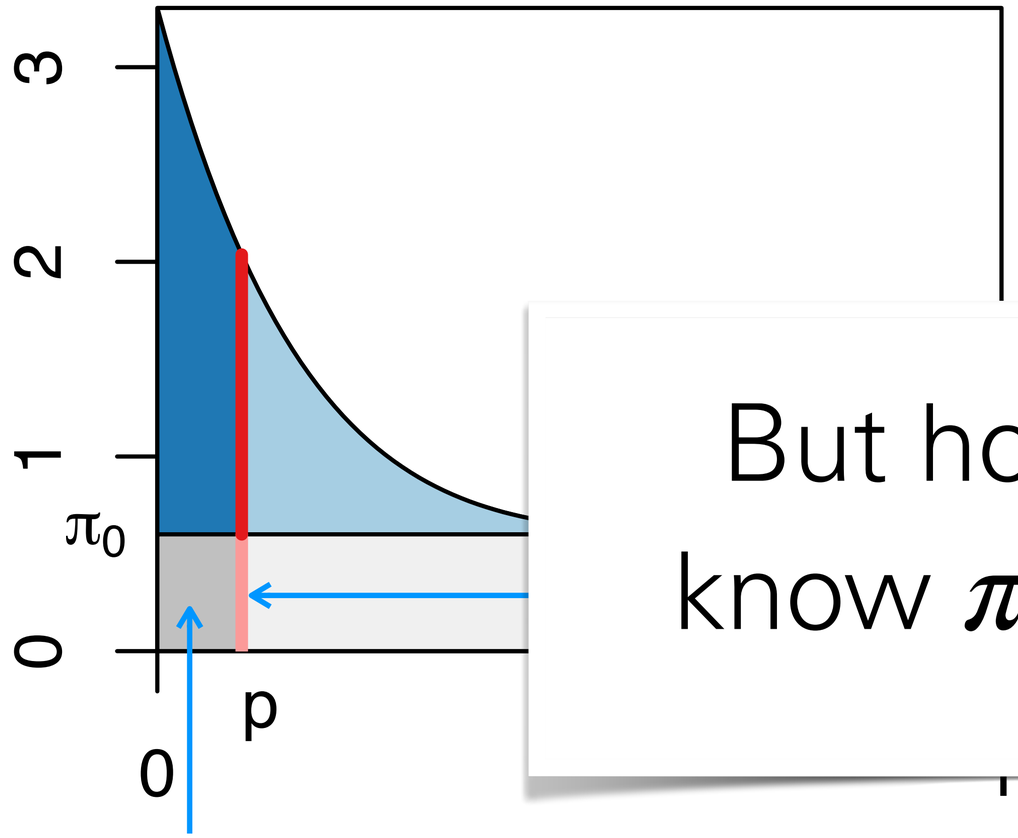
$$f(p) = \pi_0 + (1 - \pi_0) f_{\text{alt}}(p),$$

$$\text{fdr}(p) = \frac{\pi_0}{f(p)}$$

FDR: Ratio betwen the areas. An average property of all tests rejected below the threshold.

fdr: Ratio between the line segment lengths. Applies to tests rejected just at this particular threshold.

The two-groups model and the (local) false discovery rate



$$f(p) = \pi_0 + (1 - \pi_0) f_{\text{alt}}(p),$$

π_0

But how do we know π_0 and f_{alt}?

the line

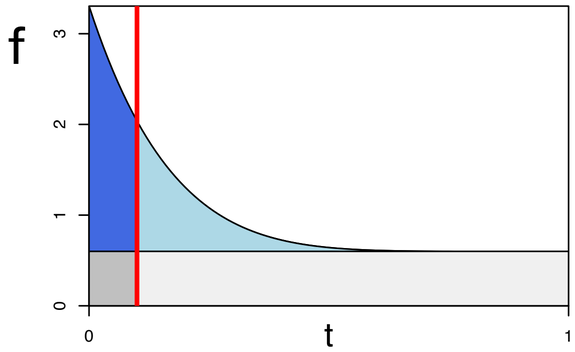
Applies

to tests rejected just at this particular threshold.

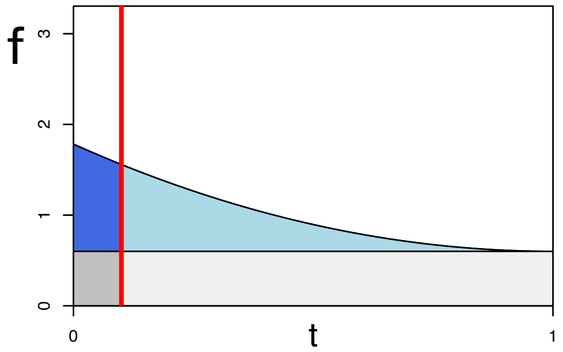
FDR: Ratio betwen the areas. An average property of all tests rejected below the threshold.

Same p-value, different FDR / fdr

$$X_i \sim \mathbb{P}^X$$
$$H_i \mid X_i \sim \text{Bernoulli}(1 - \pi_0(X_i))$$
$$P_i \mid (H_i = 0, X_i) \sim U[0,1]$$
$$P_i \mid (H_i = 1, X_i) \sim F_{\text{alt}|X_i}$$

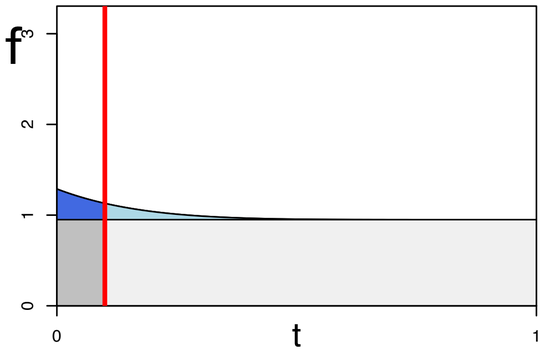


$\pi_0 = 0.6$



$\pi_0 = 0.6$

different F_{alt}



$\pi_0 = 0.95$

same F_{alt}