# Multiple Testing
# GWAS


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# simple testing

- **inference** → is there a **difference** between groups?
  - e.g. AA vs AB vs BB
- **significance** is related to the **size** and **variance** of this difference
- ***p*-value**: prob of obtaining such an extreme t statistics under $H_0$ given we repeat the experiment an infinite number of times
  - P-value < $\alpha$→ small likelihood of the data under $H_0$ → significant difference
  - P-value >$\alpha$→ there is a high chance of observing these data if there is no difference between groups
- $\alpha$ = 0.05 → threshold: 5% of rejecting $H_0$ when it is true (Type I error).
  - **false positive**: significant result when there is no difference ($H_0$ is true)

# multiple testing

- **many tests → many false positives**
  - e.g. 2000 (independent) tests, $\alpha$=0.05 → How many expected false positives?

    100 false positives by chance alone
- multiple testing problem

- A typical GWAS conducts hundreds of thousands to millions of tests independently, each for a single marker and with its own false-positive probability.
  - many SNPs, many statistical tests, many p-values (large p, small n problem)

# How to cope with the problem

1. Increase the sample size
   (e.g. Bio Banks)
2. Reduce the number of tests
   - Based on LD
   - Choose relevant regions (functional analysis)
3. Decrease the significance threshold
   - **Arbitrary significance level (**e.g. $5x10^{-8}$**)**
   - **Bonferroni correction**
   - **False discovery rate**
   - ***q* values** (*important pitfalls*)
   - **Permutation analysis**
   - **Go Bayesian...**

# Bonferroni correction

- Bonferroni, mathematician (1892 - 1960)
- **adjust** the significance threshold:
    - **New significance threshold $\leqslant \alpha$/m**

[m: number of tests (markers)]

- Bonferroni correction tends to be too conservative
    - few false positives
    - many false negatives

# False discovery rate (FDR)

- Decrease the significance threshold

0.010
0.025
0.026
0.031
0.042
0.049
0.050
0.065
0.078
0.101
0.125
0.128
...

List of ordered p-values

1) If I apply a threshold alpha to decide on significance, how much can I trust the results?
2) Where should I draw a line (threshold) of significance so that at most e.g. 10% of results are false positives?

# False discovery rate (FDR)

- **FDR:** how many of the positive results are false positives?

- Benjamini & Hochberg (1995), Storey (2002), Storey & Tibshirani (2003)

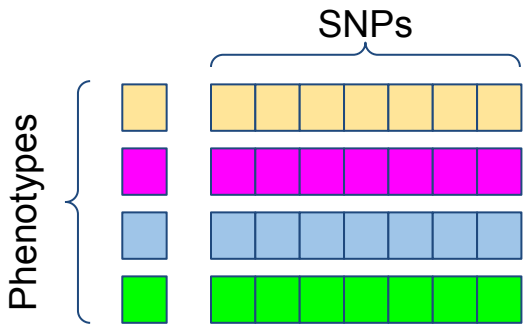
- **Significance level = 0.05** → 5% of **all** tests on average will be false positives (assuming independency)
- **FDR = 0.05** → 5% of **significant** tests will on average be false positives

fewer false positives!

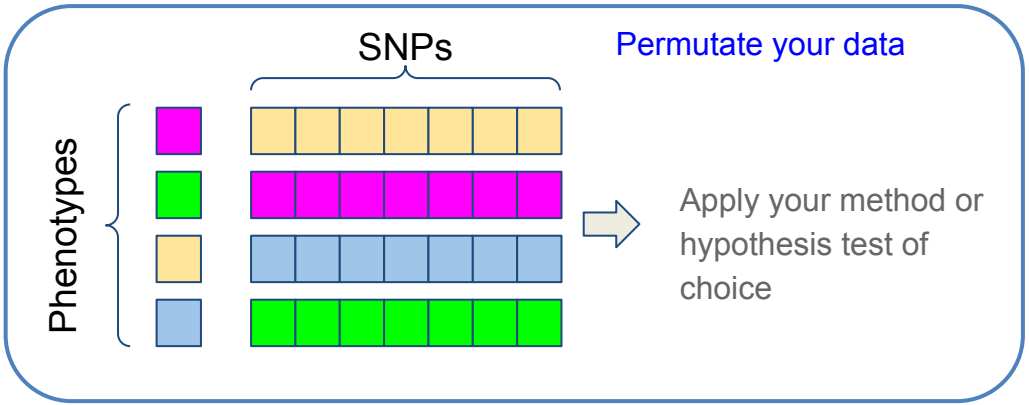
# Permutation tests

- Determine the significance of a result by randomly reshuffling the data and recalculating the test statistic.
  - This allows to test the null hypothesis that the observed difference between two groups is due to chance, rather than a real difference between the groups.
- Permutation tests are often used when the assumptions of traditional parametric tests are not met, or when the sample size is small.
- They are also useful when the data is not normally distributed, or when the groups being compared are not independent.

# Permutation tests



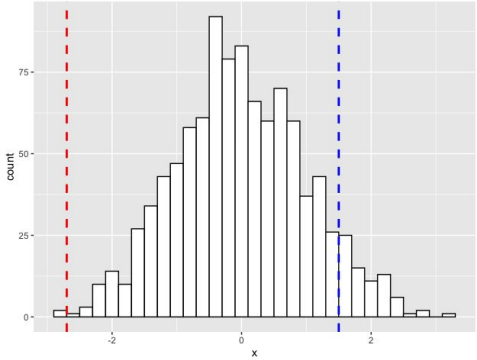

Apply your method or hypothesis test of choice




Permutate your data

Apply your method or hypothesis test of choice

x large number of times (e.g. x1000)




Non-significant, within 95%HPD

**Significant, out of 95%HPD**

## Bayesian inference

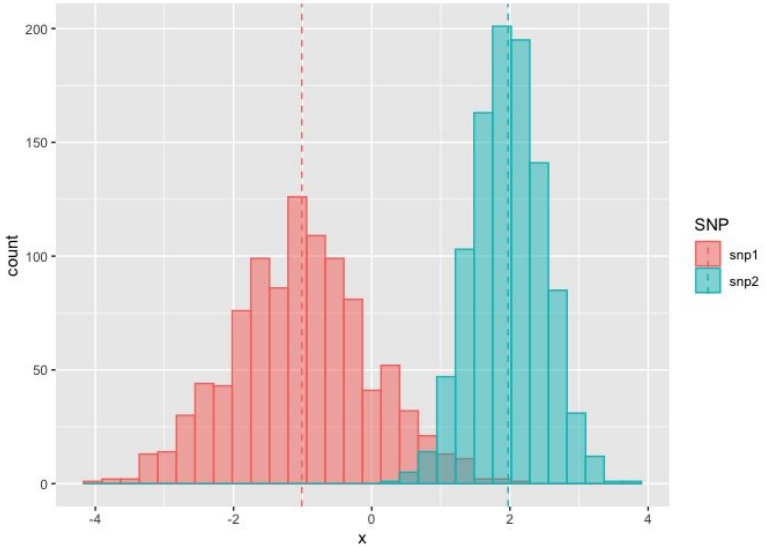
- In Bayesian inference, the probability of a hypothesis is updated using Bayes' theorem as follows

$$p(\boldsymbol{\theta}|\mathbf{y}) = \frac{p(\mathbf{y}|\boldsymbol{\theta})p(\boldsymbol{\theta})}{p(\mathbf{y})} \propto p(\mathbf{y}|\boldsymbol{\theta})p(\boldsymbol{\theta})$$

  - where P($\boldsymbol{\theta}$ |y) is the updated probability of the effect given the new evidence, P(y|$\boldsymbol{\theta}$ ) is the likelihood of the evidence given the effect, P($\boldsymbol{\theta}$ ) is the prior probability of the effect, and P(y) is the probability of the evidence.
  - Make inferences of the posterior distribution using McMC algorithms (Gibbs sampling, acceptance rejection, Metropolis-Hasting)

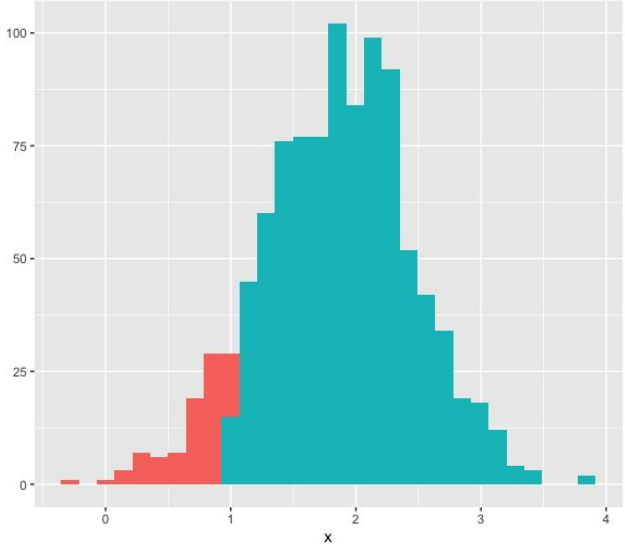
# Bayesian inference

- What is the mean of the posterior distribution and its standard deviation?
- Does it contain zero?



# Bayesian inference

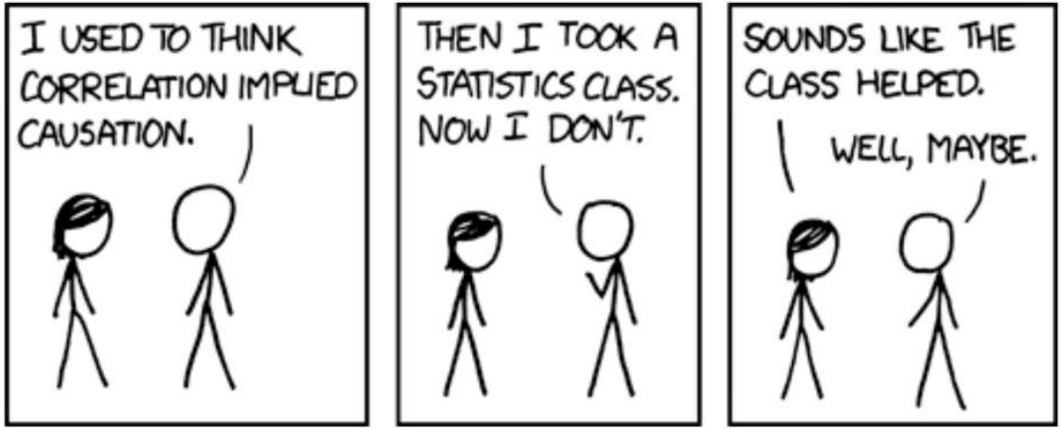
- What is the probability of the effect being larger than a relevant magnitude (e.g. +1).
- Is it a sufficient probability (e.g. 80%)




Possible to combine Bayesian inference and permutation test

# REMEMBER

- Correlation does not imply causation




https://xkcd.com/552/

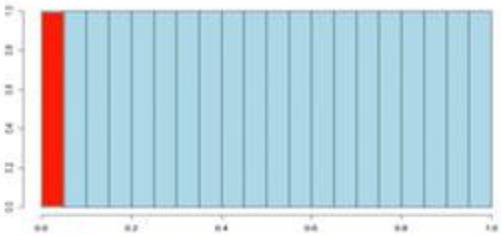
*Make your rationale choice*

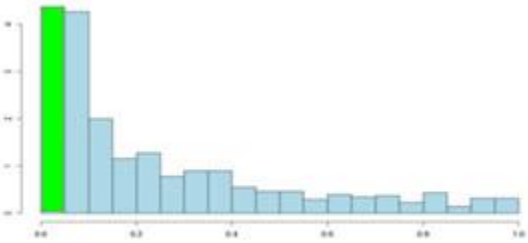
## NEXT LECTURE

# Power of GWAS experiments

# q-values

- q-values: proxies for FDR based on the **distribution of p-values**





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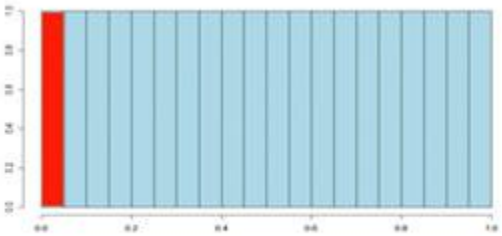
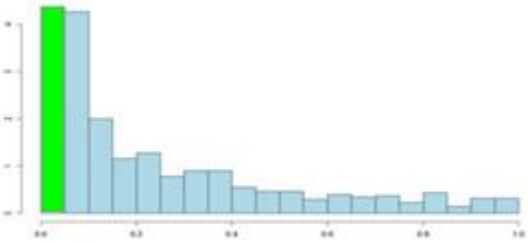


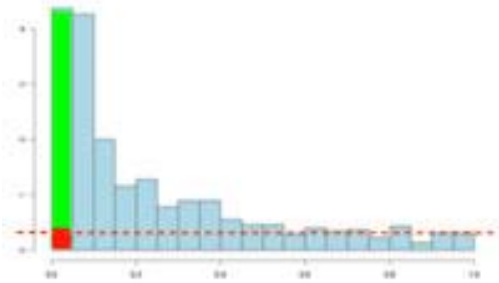


no significant differences

significant differences

# q-values

- the q-value approach tries to find the proportion of significant results which are likely to be false positives
- intuitively, it finds the height (density) at which the distribution of p-values flattens out



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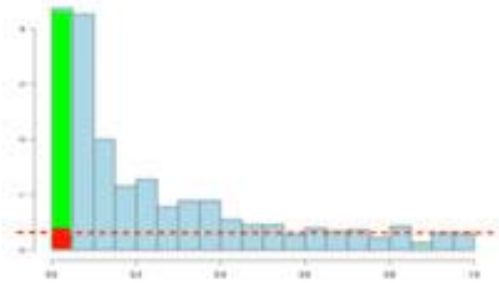

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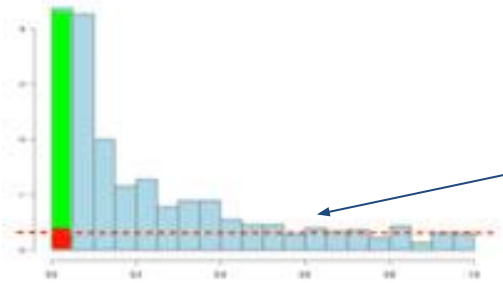

here the distribution is similar to the case where there is no actual difference

- this proportion of false positives is then incorporated in the calculation of adjusted p-values (**q-values**)

# interpretation of q-values

- *Significance level* = 0.01 → probability of the p-value under $H_0$
- q-value = 0.02 → probability of the SNP being a false positive

interpretation of the single SNP

- *Significance level* = 0.01 → 1% chance of false positives (e.g. 7900 SNPs → 79 false positives expected)
- q-value = 0.02 → 2% of positive results may be false positives (e.g. 800 SNPs with q-value ≤ 0.02 → 16 false positives expected)

interpretation of the distribution of SNPs

## q-values

- What's **wrong** with **q-values**?
  - They assume p-value is the probability of rejecting the null hypothesis when it is true
  - They do not consider that p-values are drawn from a probability distribution, and assume an infinite repetition of the experiment (obtaining different p-values for each experiment).