# Multiple testing (in R)

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# Outline for the course

- **Introduction to statistics**
- **Hypothesis testing (in R)**
- **Regression analysis (in R)**
- **Multiple testing (in R)**

# Hypothesis testing

Often, the aim of the analysis is not only **estimation**.

For example,

- *Is the treatment effective?*
- *Is a gene differentially expressed?*
- *Is there an association between genotype and phenotype?*


These questions can be translated as **hypothesis testing** problems

Comic taken from Significance Magazine, December 2008.

# Hypothesis testing

**No test is exact:**

| | **Null hypothesis does not hold** | **Null hypothesis holds** |
|---|---|---|
| **Reject null hypothesis** | Correct<br>True positive | Wrong<br>False positive |
| **Do not reject null hypothesis** | Wrong<br>False negative | Correct<br>True negative |

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For example, Genome Wide Association Studies (GWAS):

Is there an association between genotype and phenotype?

- Usually framed as a regression problem
- Outcome: disease (yes/no), biomarker value, height, etc.
- Covariates: SNPs in the genome (millions!)

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Notation:

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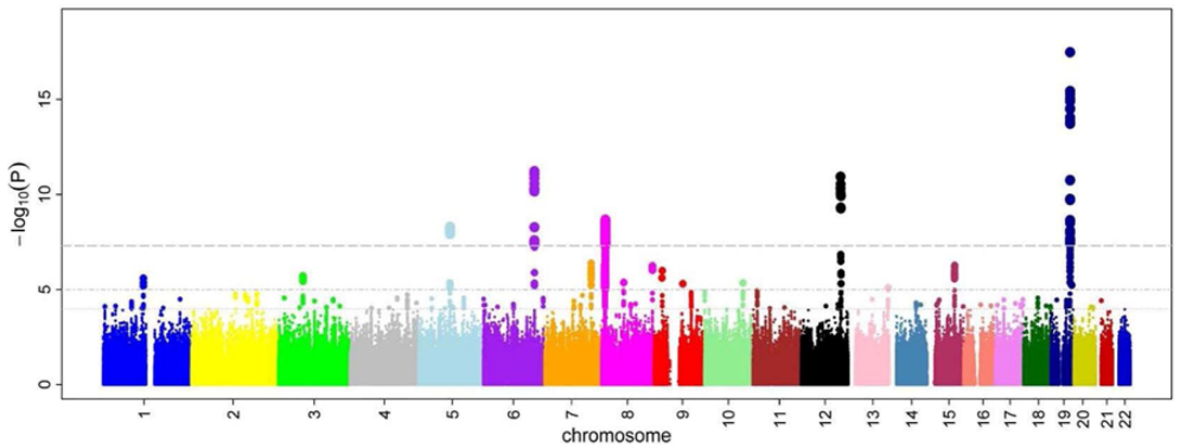


There are as many tests as SNPs in the genome!

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What happens to these errors when we run more multiple tests?

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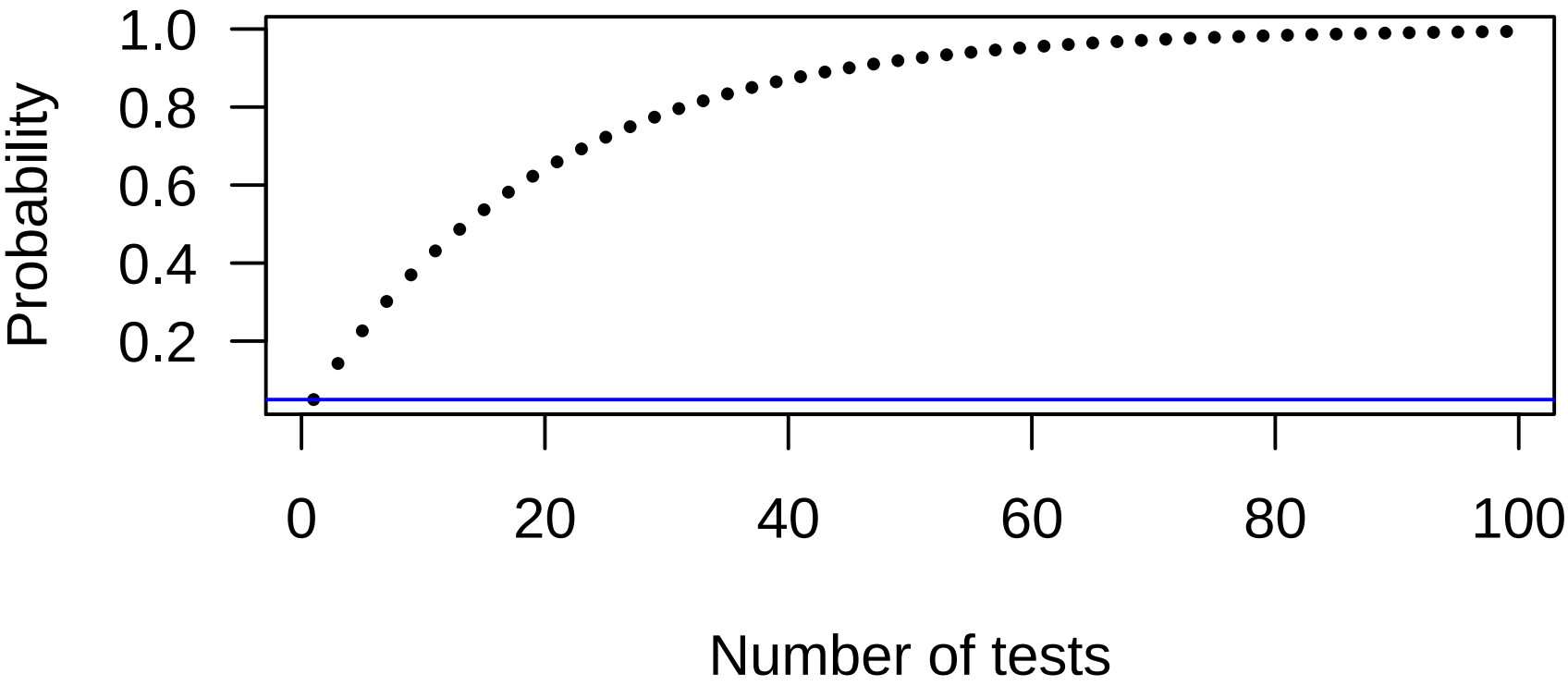
P(At least 1 FP in $T$ tests) $= 1 - (1 - \alpha)^T$; $\alpha = 0.05$




**For $T = 100$ tests, the probability of at least 1 FP is almost 1!**

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We need to correct for multiple testing to control error rates

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To control the **Family-Wise Error Rate** (FWER)

$$\text{FWER} = \text{P}(\text{At least 1 FP in } T \text{ tests})$$

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Bonferroni's solution is to use:

$$\alpha^* = \min\{\alpha/T, 1\}$$

Doing this for each test ensures that FWER $\leq \alpha$

# Multiple testing: Bonferroni's correction

Bonferroni's correction:

$$\alpha^* = \min\{\alpha/T, 1\}$$

For example, for 1 million tests and $\alpha = 0.05$, this leads to

$$\alpha^* = 5 \times 10^{-8}$$

This is commonly used as a p-value threshold in GWAS

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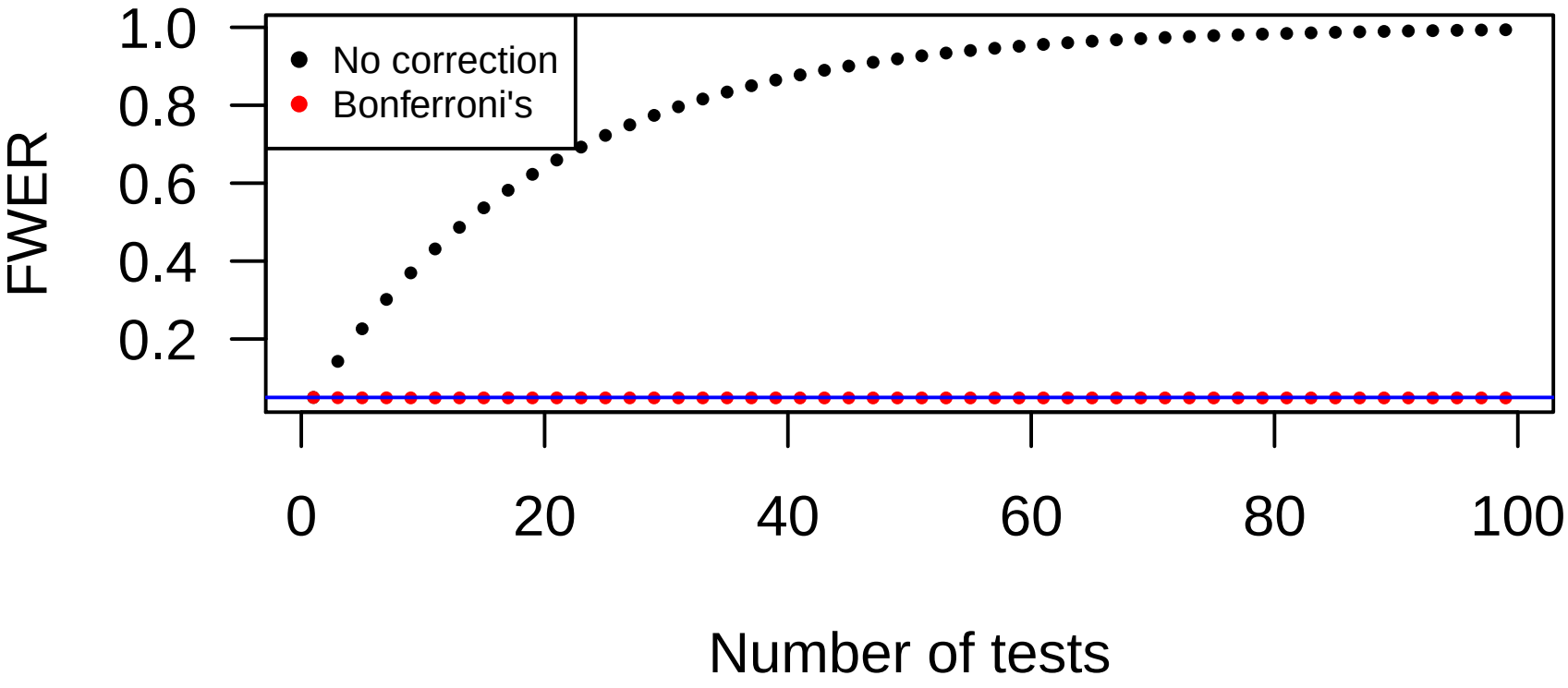

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- It can be too conservative $\Rightarrow$ too many false negatives
- Holm's method is less conservative while still controlling FWER

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**Benjamini and Hochberg's method controls the FDR**

Questions + practical