Multiple testing and the false discovery rate

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Seminar series on statistical reasoning in biomedical research

- Apr 30: P-values: What they are and what they are not (Fridtjof Thomas, PhD)
- May 07: Should We eliminate P-Values or Use More of Them: A Discussion on the P-Value Controversy (Saunak Sen, PhD)
- May 14: The Bayesian Approach to Data Analysis (Fridtjof Thomas, PhD)
- May 21: Multiple Testing and the False Discovery Rate (Saunak Sen, PhD)
- May 28: The Perfect Doctor: An introduction to Causal Inference (Fridtjof Thomas, PhD)
- Jun 04: Enhancing Statistical Methods in Grants and Papers (Saunak Sen, PhD)
Outline

Should we adjust for multiple comparisons?

Family-wise error rate (FWER)

False discovery rate (FDR)
To adjust or not?

Consider a thought experiment.

**Scenario 1:** Postdoc A tests association between gene A and phenotype; publishes paper. Postdoc B tests association between gene B and phenotype; publishes paper. Postdoc C tests association between gene C and phenotype; publishes paper. And so on, for Postdoc Y. Each paper stands on own.

**Scenario 2:** Postdoc Z uses high-throughput technology to test association of genes A-Y and same phenotype. Does postdoc Z have to adjust for multiple comparisons? Should Z be penalized for using fancier technology?
Examples

- Deviant search from mutagenesis
- Genome-wide association studies
- Differential gene expression using RNAseq
- Compare lipid profile of subjects with and without statins
- Compare microbiome of obese and healthy individuals
Family-wise error rate (FWER)

Appropriate when multiple tests are used to test a single hypothesis.

- Bonferroni procedure
- Holm procedure
- Fisher combination procedure
Bonferroni procedure

If we have $m$ ordered p-values $p_1 \leq p_2 \leq \ldots \leq p_m$, then the FWER corrected p-value is $m \times p_1$.

For example, if we perform three tests and get p-values 0.1, 0.04, and 0.01, and we want to control FWER to be 5% or less, then we will reject the null hypothesis only if the smallest p-value is $0.05/3$ or less. In this case, one p-value (0.01) is smaller, and therefore we can reject the null hypothesis controlling the FWER at 5%. The Bonferroni-corrected p-value of the family of tests is $0.01 \times 3 = 0.03$.

- Simple and easy to conduct
- No assumption on dependence between tests
- Low power if $m$ is large
Fisher combination procedure

Suppose we have \( m \) p-values \( p_1, p_2, \ldots, p_m \) from independent tests. Let \( T = -2 \sum \ln(p_i) \). This is called the combination statistic.

Compare this to a \( \chi^2 \) distribution with \( 2m \) degrees of freedom to get the combination p-value for the family of tests.

In the example considered above the combination statistic is \( T = 2(2.30 + 3.22 + 4.61) = 20.26 \) which gives a p-value of 0.0025 when compared to a \( \chi^2 \) distribution with 6 degrees of freedom.

- More complex than Bonferroni
- Assumes independence of tests
- More power than Bonferroni
- Weights p-values equally regardless of information content
False discovery rate (FDR)

Appropriate when we want to evaluate a large number of similar hypotheses individually.

We want to estimate the probability that a “discovery” is false, given that we have a discovery (low p-value).

Note similarity with diagnostic tests: we want to estimate the chance that a discovery (positive test result) is false (from a normal sample).
P-value distributions under null, alternative, and observed

Analogy with diagnostic tests: The diagnostic test is based on the p-value. The characteristics of the diagnostic test under null (normal sample, $F_0$) is known, and the alternative (diseased sample, $F_1$) is partially known. The disease prevalence $(1-\pi_0)$ is not known.
Actual and declared true/false hypotheses

<table>
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<th>Actual</th>
<th>Declared</th>
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<tr>
<td>Total</td>
<td>$W_t$</td>
<td>$R_t$</td>
<td>$m$</td>
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Two approaches to FDR

There are two main approaches. The first, due to Benjamini and Hochberg seeks to find a cutoff, $t$, given a target proportion $\alpha$, such that

$$E \left( \frac{V_t}{R_t}, R_t > 0 \right) \leq \alpha.$$ 

The second, due to Storey, and closely related to that of Efron and Tibshirani, seeks to find the $q$-value, for a fixed cutoff $t$ such that

$$q_t = P \left( \frac{V_t}{R_t} | R_t > 0 \right).$$

Thus, the first approach estimates a cutoff given a target $FDR$; the second estimates the $FDR$ given a cutoff.
False discovery rate (FDR) given cutoff (q-value)

The q-value, or FDR corresponding to a cutoff of \( t \) is the probability of a false discovery given that there has been a discovery (a p-value under the cutoff).

We can use Bayes theorem for that.

\[
P(H_0|T \leq t) = \frac{P(H_0)p(T \leq t|H_0)}{P(T \leq t)}
\]

\[
q(t) = \pi_0 F_0(t)/F(t),
\]

where \( F = \pi_0 F_0 + \pi_1 F_1 \). We can estimate \( F(t) \) from the empirical distribution of p-values, and \( F_0(t) = t \) since \( F_0 \) is uniform. Thus

\[
\hat{q}(t) = \pi_0 t/\hat{F}(t) \leq t/\hat{F}(t).
\]

Sharper estimates possible by estimating \( \pi_0 \).
Get cutoff given desired False discovery rate (FDR)

Let \( p_1, p_2, \ldots, p_m \) be the ordered p-values. If \( \pi_0 \) is the proportion of null hypotheses, then the cutoff \( t \) such that

\[
E \left( \frac{V_t}{R_t}, R_t > 0 \right) \leq \alpha
\]

is

\[
t = \max \left\{ p(i) : p(i) \leq \left( \frac{i}{m} \right) \left( \frac{\alpha}{\pi_0} \right) \right\}.
\]

In practice, we do not know \( \pi_0 \), so a conservative choice is \( \pi_0 = 1 \).
Benjamini-Hochberg procedure

\[ \text{Cutoff} = \frac{\alpha}{\pi_0} \]

- p-value
- quantile
- Cutoff

\[ \alpha \]
Battery of 6 tests (vertical movement, ambulatory movement, RER; in dark and light phases) per mouse. A FDR of 10% was desired.

<table>
<thead>
<tr>
<th></th>
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<th>Number Tests</th>
<th>FDR Mice</th>
<th>FDR Tests</th>
<th>P-value Cutoff</th>
<th>Bonferroni Mice</th>
<th>Bonferroni Tests</th>
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<td>28</td>
</tr>
</tbody>
</table>
Gene expression example

Compare genomewide gene expression between two mouse strains in spinal cord tissue samples. The Illumina mouse arrays that we used can hybridize up to six samples on the same “chip”. Spinal cord mRNA from 6 B6 mice, and 6 LP/J mice. Two chips were hybridized. In each chip mRNA hybridized to three B6 and three LP/J samples. A total of 48358 probes. Our goal is to find out which genes (probes) are differentially expressed between strains.

If we perform a Bonferroni correction to the p-values, then only genes with p-values smaller than $0.05/48358 \approx 10^{-6}$ would be considered significant. Only about 654 genes fit this bill.

However, if we estimate the q-values, and find genes with a q-value smaller than 0.05, then we find 4163 genes. If we look for genes with a q-value smaller than 0.01, we find 2459 genes. An estimated 27% of the genes show differential expression.
P-value distributions

- P-value for strain effect
- P-value for chip effect

Graphs showing density distributions for p-values.
Using permutation

An alternative analysis would be to use a permutation test, by permuting the strain labels *within* each chip. The permutations allow us to obtain the distribution of the test statistic under the hypothesis of no strain effect, but in the presence of a possible chip effect. In this data, we have only \( \binom{6}{3} = 20 \) permutations possible. By combining the permutations in both chips, a total of 400 permutations are possible. We exhaustively enumerated each of them and calculated the average strain effect within chip. This was then compared to the observed average strain effect within chip and ranked.

For 1761 probes the observed effect was ranked 1, and for 1961 probes it was ranked 400. Thus the two possible extreme ranks were observed 3722 times compared to the expectation of \( 48358/200 \approx 242 \) under the null. Thus the genes with rank 1 or 400 have a q-value of at most \( 242/3722 \approx 0.065 \).
P-value distributions from permutations (exhaustive enumeration)

Figure 1: Rank of observed effect among permutations of strain labels within chip. The effect was measured as the average strain effect within chip.
Given a cutoff, \( t \), the q-value is the proportion of null hypotheses among the discoveries. It is one minus the positive predictive value.

The p-value under the null must be correctly calibrated, otherwise the FDR estimates are incorrect.

The p-value distribution under the alternative is not needed, but it is assumed that small p-values are more likely under the alternative.

Estimates of the proportion of null hypotheses \( \pi_0 \) affects the q-value (and FDR), just like the disease prevalence affects the positive predictive value.

Independence of the tests (p-values) is not required as long as we can get good estimates of the p-value distributions.

There is an implicit understanding that the hypotheses/tests are exchangable (or similar).
Consider research question carefully to decide if multiple comparisons adjustment is needed, and if so, what kind

- FWER appropriate when many tests are used to test a single hypothesis
- FDR appropriate when we want to evaluate a large number of similar hypotheses individually

- Be transparent regarding process and assumptions
- Report all p-values so that others can replicate or adjust assumptions
Further reading


R functions:

- `stats::p.adjust`
- `qvalue::qvalue`
Upcoming

▶ May 28: The Perfect Doctor: An introduction to Causal Inference (Fridtjof Thomas, PhD)
▶ Jun 04: Enhancing Statistical Methods in Grants and Papers (Saunak Sen, PhD)

Slides at https://tnctsi.uthsc.edu