BONFERRONI CORRECTION

When we make a hypothesis test, like comparing the differential expression of genes in a disease, we will always identify some genes related to the disease, even if none of the genes are linked.

For example, in a DNA microarray where 3000 genes are tested, considering the confidence level, the p-value of 0.05 will wrongly identify 150 genes as related to the disease.

Here we are committing the type I error. This error is when you reject the null hypothesis when it is true, also known as a false positive.

In the previous example, the hypothesis would be:

- H_o (null hypothesis): no differential expression of the gene.
- H_a (alternative hypothesis): differential expression of the gene.

We consider these genes wrongly defined as statistically significant and related to the disease. False positives typically appear when multiple hypothesis tests are performed at once. The probability of identifying at least one significant result due to chance increases as more hypotheses are tested.

This is known as the Family-Wise Error Rate (FWER), the probability of making one or more false positives or type I errors when performing multiple hypotheses tests.
It can be calculated as:

$$FWER = 1 - (1 - \alpha)^n$$

Where α is the significance level for a single hypothesis test, and n is the total number of tests.

If we consider a significance level of 0.05 and 1, 10, and 20 genes compared at once, we can see how the probability of committing the type I error increases as it increases the number of tests.

$$FWER = 1 - (1 - 0.05)^1 = 0.05$$

$$FWER = 1 - (1 - 0.05)^{10} = 0.40$$

$$FWER = 1 - (1 - 0.05)^{20} = 0.64$$

To minimize this error, we have to adjust α to the experiment and reduce the chances of obtaining a false-positive. This is carried out with the Bonferroni Correction.

Bonferroni Correction is an adjustment to p-values when several dependent or independent statistical tests are performed simultaneously on a single data set. This will lead to a lower probability of committing type I error.

It is the result of the significance level (α) divided by the number of comparisons being made:

$$\alpha_{adjust} = \frac{\alpha}{n}$$

In the example of the DNA microarray, the alpha would be:

$$\alpha_{adjust} = \frac{0.05}{3000} = 1.67e^{-5}$$

$$FWER = 1 - (1 - 1.67e^{-5})^{3000} = 0.0488$$

The significance level for the experiment changes from 0.05 to $1.67e^{-5}$. This way, we can maintain the Family-Wise Error Rate at a 5% level. With this lower significance level, fewer genes would be considered statistically significant when they are not.

However, the Bonferroni Correction has a disadvantage. As the number of simultaneous comparisons increases, it also increases the probability of generating false negatives, accepting the null hypothesis when the alternative is true. This is a Type II error.

This occurs because Bonferroni Correction is a very conservative method. Type I errors cannot decrease without inflating type II errors. And type II errors are no less false than type I errors.

The Bonferroni Correction is a simple statistical method for mitigating this risk, but it should not be performed routinely as it is too conservative and applies the correction

uncritically. So its use depends on the circumstances of the study as it does not always guarantee a correct interpretation of results.

The method is sometimes used in many meta-analyses to understand the results better.

An example is Gao J et al. Association between IL-6-174G/C Polymorphism and the Risk of Sepsis and Mortality: A Systematic Review and Meta-Analysis. 2015;10(3). (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4348480/)

The study discusses that recent studies have reported an association between IL-6-174G/C polymorphism and sepsis. However, the results are inconclusive and conflicting. To better understand the role of IL-6-174G/C polymorphism in sepsis, a new analysis is conducted using the Bonferroni correction.

Although there was a statistically significant association between IL-6-174 G/C polymorphism and sepsis-related mortality under the recessive model, the significance did not exist after Bonferroni's correction.

With this result, we see how data could be misunderstood, and the correction of the p-values is a way to deal with this error.

For the theoretical justification of the Bonferroni Correction, you could check the following document (pg. 41):
http://eio.usc.es/eipc1/BASE/BASEMASTER/FORMULARIOS-PHP/MATERIALESMASTER/Mat_12_APUNTES.PDF

Or a different explanation in the next video:
https://www.youtube.com/watch?v=HLzS5wPqWR0