Biotelligences Fortnight

Issue 2 (June 23 2014): Ramasamy et al. Nature

Notch for bones and vessels

Biotelligences Fortnight aims to showcase a recently published high impact article chosen by us, based mostly on the quality of experimental design, statistical analysis and presentation. Biotelligences Fortnight is released every two weeks or so in the form of a summary that highlights the major points that have appealed to us in the article as well as possible points that we think could have been improved. It is not an all-inclusive collection of statistical details but rather a guide for your own research. We are happy to receive suggestions from your recent reading.

This week, we selected a short article from Ramasamy and colleagues published in *Nature* on March 20 2014 (PMID: 24647000). The authors elegantly use a series of genetic and molecular approaches to suggest that, as opposed to its previously described anti-angiogenic actions, the Notch pathway has a pro-angiogenic influence in bones, which in turn stimulates osteogenesis. Although not flawless, the high standards of the biostatistics in this article should be seen more often in high impact journals, and in *Nature* in particular. We liked: (1) the quality of the statistical paragraph, which recapitulates the authors' policies about experimental blinding, randomization and outliers as well as disclosures of the alpha threshold (0.05) and statistical software used; (2) the corrections made for multiple comparisons in Fig 3 and 4 using one-way analysis of variances (ANOVA) followed by Bonferroni tests. The Bonferroni correction is very conservative (reduces the chances of detecting an actual significant result) compared to other corrections, so the observed effects are very likely due be real; (3) the disclosure of exact p-values and sample sizes in figures and legends, which allows the reader to critically discuss the results.

One special mention has to be made about the presentation and discussion of Fig 4a, where the authors conclude that the contents of various mRNAs are increased, although only one (*Nog*) shows a significant (p=0.0369) augmentation. No claim is made about any "statistically significant" change, therefore the conclusions of mRNA increases are valid. Way too often in publications (actually almost invariably) researchers only rely on p-values to conclude, ignoring the effect size (strength of the effect) and real meaning of the p-value (chance to erroneously conclude of an absence of difference).

However, we think that data presentation may be improved. The inaccurate use of standard error (SEM) to show variability in Fig 1j, 1k, 2f, 2k, 3e, 4a, 4b and systematic use of bar graphs are misinforming. Instead, we suggest displaying individual values with single dots together with standard deviation for variability. In addition, the experimental power may generally be too low, probably resulting in the lack of significance in Fig 4a. Finally, there are apparent possible violations of parametric assumptions (unverified normality, possible unequal variances) in several data sets (Fig 1j and 1k).

In conclusion, despite several flaws, the biostatistics in this article are of very good quality.

The Biotelligences team

Authors' comment:

"We are very pleased that our research article is being highlighted by Biotelligences. While the amount and complexity of biological research data are rapidly increasing, sample size in studies involving animal models is limited by factors such as availability, costs and, most importantly, ethics. The valuable comments and suggestions provided by the biostatistics experts will surely facilitate the design of future studies"

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