Response to Letter Regarding Article, “Late Sodium Current Inhibition Reverses Electromechanical Dysfunction in Human Hypertrophic Cardiomyopathy”

We thank Sikkel et al for their appreciative letter, particularly focusing on the statistical approach used in our recent work. Indeed, physiological parameters of cells from human subjects exhibit considerable and intrinsic interindividual variability, at variance with inbred rodents. Thus, when working on human data, the use of hierarchical statistical analysis is not only the most valid option, but one could say it is mandatory to perform reliable comparisons between normal and diseased states. Hierarchical statistical analysis has been used previously in biomedical research involving human subjects, including a recent study comparing mechanical data from single myofibrils isolated from cardiac samples of patients with hypertrophic cardiomyopathy with donor hearts and secondary hypertrophy. However, this approach has not been used previously in the field of cellular cardiac electrophysiology, where standard statistical methodologies have been classically used.

Hierarchical analysis should also be considered strongly in studies involving animal models, especially when experimental surgery or other manipulations are involved, because the outcome of such operations is likely dependent on the individual response of each animal, as well as on the technical success of each procedure. We were asked recently to take into account the variability among animals while using a rat model of heart failure after myocardial infarction, albeit recently to take into account the variability among animals while using a rat model of heart failure after myocardial infarction, albeit with the use of a different statistical method.

In our recent work on human hypertrophic cardiomyopathy, we performed a complete sensitivity analysis for each statistical comparison, including the following steps:

1. verifying whether the data followed a Gaussian distribution using the Skewness/Kurtosis normality test;
2. verifying whether the variances within the 2 groups were equal using the F test for equality of variances in 2-group comparison studies and the Bartlett test for variance homogeneity in the multiple comparison design;
3. using a nonparametric test on the basis of rank transformation (Wilcoxon sum of rank) to check robustness of results if conditions 1 or 2 were not met (ie, when data were not normally distributed or variances were unequal);
4. estimating within-subject correlation for each variable using 1-way ANOVA;
5. using linear mixed models to compare couples of data groups, both paired and unpaired, to account for the correlation among different cells or muscles from the same patient; correction for heteroscedasticity (inequality of variances) was applied to linear mixed models in unpaired comparisons whenever the variances of the 2 groups were unequal (as calculated by the F test in step 2).

Of note, significant discrepancy between standard t test analysis and hierarchical analysis was found when within-subject correlation (as calculated in step 4) was high, emphasizing the inadequacy of classic statistical methods in this setting.

In conclusion, we share with Sikkel and colleagues the auspice that hierarchical statistical methods will be publicized and become of common use, as a permanent adjunct to the toolbox of basic and clinical scientists in cardiovascular research.

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