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LETTER TO THE EDITORS

Reply to the "Letter to the Editors" by Steven Buyske

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Dear Editors:

We appreciate the opportunity to clarify the statistical treatment of our data. During the preparation of the manuscript, we did consider the use of the *t*-test as proposed by Dr. Buyske. Indeed, the *z*-test requires that the standard deviation be known, while we estimated the standard deviation from the sample. However, the *t*-test is not ideal either. A *t*-test compares two groups of values and the statistics rely on the standard deviation of both groups. It is unconventional to use the *t*-test to compare a single value, i.e., that of the miRNA in each case of autism against a group of values. By definition, the single values do not have a standard deviation. For this reason, we chose the *z*-test as a first-level less stringent screen to assess differential expression between one autistic case and 13 normal cases for each miRNA.

We had also addressed the issue raised by Dr. Buyske with a nonparametric approach—the Wilcoxon rank test. However, the statistical power of the rank order test is rather weak and, more importantly, we lose the information in the differences in magnitude of the miRNAs. A strength of the method we used to profile the miRNAs—real-time PCR—is its ability to detect a broad dynamic range of miRNA expression. Our method was preferable to capture this quantitative parameter. However, the miRNAs with *p*-values that rank among the top using the parametric test remain among the top with the nonparametric test.

The statistical testing as suggested by Dr. Buyske does demonstrate some differentially expressed miRNAs. With a one-sided *t*-test, our top five miRNAs remained statistically significant. The main difference between the *z*-test and *t*-test in a sense is the *p*-value cutoff. The *z*-test is indeed less stringent than the *t*-test and the two-sided *t*-test further increases the stringency. On the other hand, the Bonferroni correction for multiple hypotheses testing, applied in the manuscript, is often considered overly stringent. If we control for multiple hypotheses with a 5% false discovery rate (FDR) rather than the Bonferroni correction on *t*-statistics, the number of dysregulated miRNAs goes from five to 13. One could further argue that even the FDR is overly stringent because many miRNAs are co-regulated, and therefore treating each as an independent query may be unnecessarily conservative.

Whichever the test and correction, we did provide all *p*-values signifying the difference between each autistic case and the normal cases for each miRNA, and these *p*-values are not affected by the significance cutoff. These *p*-values

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allow the reader to see all the data and apply their own cutoff as Dr. Buyske has done.

We think that our approach, *z*-test (less stringent than *t*-test) plus Bonferroni correction (more stringent than FDR), as well as the inclusion of all the raw data in our manuscript provides researchers a better opportunity to explore the effects of miRNAs on autism. Some validation of the results appears in our paper. We reported that, among the implicated miRNAs, several target genes known to be involved in autism are predicted. Furthermore, four of the miRNAs we reported (miR-23a, miR-132, miR-146b, and miR-320) were reported in a subsequent study using lymphoblastoid cell lines from six subjects with autism and six controls (Talebizadeh Z et al., *Autism Research* 1:240–250, 2008).

We thank Dr. Buyske for his comments and the opportunity to better convey the findings.