Reviewer’s report

Title: Reduced serum levels of pro-inflammatory chemokines in Fragile X Syndrome

Version: 0 Date: 30 Oct 2019

Reviewer: Quang Minh Bui

Reviewer's report:

Summary:

This paper is concerned about possible immune dysfunctions in Fragile X (FXS) syndrome by examining 52 immune-related biomarkers (cytokines/chemokines) in the FXS patients and compared to that of the healthy controls. The application of K-sparse clustering algorithm to discriminate FXS status is novel. However, my major concerns are that the study also included females (5 in each group), which previous two studies by Ashwood et al. and Careage et al. did not, and the effect of outliers was not carefully examined, see more detail in comments below.

General comments:

1. Since the sample size in this study was small the number of bootstraps of 200 may not be enough. Could the authors re-run analysis with more runs, e.g 2000, to see if results would be difference?

2. As the number of females is small in each group, it would be better to give p-value in the Table 1 using Fisher's exact test.

3. The use of elastic net logistic regression and K-sparse algorithm for clustering analysis in R require a special program to conduct analyses. If the programs were not written by authors, it would be fairs for other researchers, who wrote the programs, that the authors would acknowledging their works here.

4. Table S1 contained summary statistics, effect size and FDR adjusted p-value and the Table S2 contained information about which biomarkers were accepted or rejected. Should Table S1 be Table S2 and vice versa, see lines 19-26, page 9? From here on, I will prefer the corrected table labeling as in the text.

5. In the Figure 1, the boxplot for CCL22 gave the range values from 0 to 2.0, but in the Table S2 the medians were 991.1 and 318.1 respectively for controls and FXS. Why?

6. Were the biomarkers dependent on age, gender, BMI and time of sampling? If they did not, the non-parametric Mann-Whitney test used for comparison between two groups in the Table S2 is robust against outliers, therefore results in this table are OK. Otherwise, the comparisons should be conducted adjusting for these covariates. This can be performed using robust regression, the method that can downweight the effect of outliers, such those ones that observed in boxplot in the Figure 1.

7. Again, about the outliers, the elastic net regression does not handle outliers (like robust regression for continuous outcome data) and consequently could affect the results. There is a possibility these outliers may come from the same two or three individuals. If this is the case, the authors should exclude...
them from analysis.

8. As shown in the Table 1, sex, age, BMI and time of sampling were not significant difference between controls and FXS, therefore, controlling for them in the elastic net logistic regression was not necessary.

9. There were no comparisons between males and females for the biomarkers and therefore difficult to know whether the expression level for biomarkers were the same in both sexes. There may be possibility that males and females express differently. In both studies cited by authors, i.e Ashwood et al. and Careaga et al., no females were selected. Therefore, it would interested for readers to see analysis for Table S2 and Table 2 replicated for males only.

10. The authors comments that the misclassification of individuals (line 13, page 11) appeared to be due to outliers, but did not shown how. There is an R package called "mvoutlier" that can be used to detect outliers in multivariable data. If there are outliers and these are excluded, I would be interested to see if classification can achieve 100%.

11. For the Table S6, it would easier for readers to read results in matrix form with the correlations present in upper triangle and corresponding p-values in the lower triangle, with empty cells for diagonal. Note that the current table format has correlation statistics for a variable with itself, which should be not be presented and should use NA instead.

12. From the Figure 3, there are moderate to high correlations among selected biomarkers. It would be interesting to obtain composite scores, such as first and second principal components, and then performs analysis to see how strong these scores associated with FXS status.

Minor comments:

7. Line 30, page 9. P-value for CCL3 should be 1.35x10^-3, not 1.53x10^-3.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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