Reviewer’s report

Title: Gene expression profiling in patients with polymyalgia rheumatica before and after symptom-abolishing glucocorticoid treatment

Version: 0 Date: 13 Mar 2017

Reviewer: Emily Camilleri

Reviewer's report:

The authors have performed a study to evaluate the underlying gene expression patterns of muscle tissue from PMR patients and controls, before and after glucocorticoid treatment. Overall, the study design was well thought out and the approach for interrogating differences was very appropriate. The results of the gene expression microarray provided novel insight into the transcriptional changes that occur in patients with PMR suggesting deregulation of protein synthesis. It was also particularly interesting to see transcriptional changes following prednisolone treatment which suggest restoration of the expression of proteins important for myocyte function. Whist these findings are of significance, there are significant concerns about making further conclusions as the methods describe the storage of RNA at -20°C. Following isolation of RNA, samples should be minimally stored at -80°C to slow down the rate of degradation. This may in part explain the lack of concordance of the qPCR with microarray results. Therefore, whilst the results of this study can make a contribution to the field, further work is require to ensure the validity of the results.

Major revision:

- Re-extract RNA and analyze the expression of candidate genes by qPCR, to validate the findings of the microarray.

Below are minor comments to consider:

Page 2, Line 34: Sentence should read: Prednisolone normalized erythrocyte sedimentation rate and C-reactive protein in PMR patients.

Page 3, Line 54: To which population are you referring? The age group or a specific ethnic population?

Page 3, Line 56: Please check manuscript for consistency of abbreviations for ESR, CRP, cDNA.
Page 3, Line 59: Please expand upon the sentence that PMR reflects synovial inflammation.

Page 4, Subjects section: This section could be more concise, as it is not necessary to thoroughly describe the information provided in Table 1.

Page 4, Line 96: Sentence repeated from above line 93.

Page 4, Line 98: Sentence should read: …exclusion criteria described by Kreiner and colleagues.

Page 5, Line 117: -80°C is sufficient.

Page 6, Line 127: It is preferred to store isolated RNA at -80°C to prevent degradation and therefore there is significant concerns about the integrity of the RNA samples used for downstream analyses.

Page 6, Line 133: Please include a table or comment about the integrity of the RNA.

Page 8, Line 178: This section could be more concise, as it is not necessary to describe what the functional annotation does. It would be reasonable to delete the remainder of the sentence "this process associates…", and more briefly describe enrichment values.

Page 8, Line 186: Please be aware that the enrichments scores strongly depend on the number of genes uploaded into DAVID. Therefore, small gene lists do not generally get very high enrichment scores. With that in mind, there may still be biological relevance for categories with lower enrichment scores.

Page 8, Line 198: delete de novo

Page 9, Line 202: Table 6 - Primer sequences was not included in the submission, and also there is a conflict with the results section which also describes a Table 6 - with qPCR vs microarray results.

Page 9, Line 207: What type of analysis of the qPCR data was performed? A relative comparison? If so was RPLP0 used as the internal control? Why was basis of selecting RPLP0 as the control?

Page 9, statistics: What statistics were used to compare ESR and CRP levels between patients and controls?

Page 9, statistics: Student's t-test are not the ideal test to be performed for this type of data due to the small sample size. Perhaps analyze the data instead using a non-parametric method such as Wilcoxon/Mann-Whitney U.

Page 10, Line 226: This section is unnecessary and can be summarized in the first paragraph of the following section.
Page 10, Line 238: For all section headings, re-word them so they are a little more direct. For example, "Differential expression of genes in PMR patients and controls".

Page 10, Line 241: The layout of Figure 1 makes it difficult for a reader to interpret.

Page 10, Line 244: For Figures 2, 3, and 4 the GO categories are small and difficult to read. Perhaps the GO number and increase the text size.

Page 10, Line 244: Was the entire gene list submitted for functional annotation analysis? Typically, analyses are performed separately for genes that were upregulated and downregulated. Although, I do understand the limitations with the small gene list.

Page 10, Line 246: "data not shown" rather than "not shown in figure 2"

Page 12, Line 293: Table 5: It would aid readers by adding a row above the fold changes that describe the comparison. It can be come tedious to look at all the symbols and read the description below.

Page 14, line 340: Please remove "Insert FIG 1" and others from this section and only refer to the figures in the text.

**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.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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