Reviewer’s report

Title: A longitudinal study of gene expression in healthy individuals

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Reviewer: Halima Moncrieffe

Reviewer’s report:

This study provides gene expression data from peripheral blood samples of healthy adult volunteers stratified by age and gender. This provides a useful data resource comparing qPCR and Affymetrix HG U133 Plus 2.0 microarrays. Blood was sampled at 5 time points, taken in the morning, post-fasting over a 6 month period. 28 individuals were sampled for Affymetrix arrays and a number of inflammatory genes were selected for qPCR.

MINOR ESSENTIAL REVISIONS:

1) P.5, If there is data for ethnicity of volunteers, this should be provided.
2) Given that this is a longitudinal study, samples will have necessarily had differing times in storage. The authors should clarify how much time the sample spent as RNA and where it was stored until it was processed for microarray. E.g. were all samples stored in the Paxgene kit and then extracted just prior to processing for microarray?
3) P11 – how were the subset of 28 individuals chosen?
4) Figure 1: Y-axis is obscured.
5) Figure 3: 2 probe sets have fold change less than 0.5 and have a p value above the nominal Type I error cutoff. The text should clarify that this figure is prior to multiple testing correction.
6) P14: Due to microarray processing in two batches, it was not possible to compare the gene signal intensities over 6 months as perhaps implied in the abstract text, however the microarray data was compared for d90 to d180 i.e. 3 months and up to day 28 ie 1 month.
7) Figure 7: Y-axis is obscured.
8) Additional file 1 needs a figure legend and to crop off the small print at the bottom of each histogram.

DISCRETIONARY REVISIONS

1) p6. RIN <6.5 is a low threshold. What was the average and range of RIN for those samples which were included in the study?
2) Why was an UNG digest necessary for this qPCR reaction.
3) P7. 2 ng of RNA, easier to follow than two ng of RNA

4) P8 Reference for SAS Proc Mixed.

5) P9 Quote total number of microarrays used, if present in at least one sample ie 1 of 112?

6) P12 – do dotted lines refer to 95% Confidence intervals on additional file 1. Figure legends should be provided for the additional files.

7) To clarify conclusion point 2, p19. Do the authors have data showing RNA extraction and storage using different Pax gene kit lot numbers and then extraction for gene expression profiling. I suspect the authors mean the batch effect on Affymetrix processing rather than RNA extraction, but this is not clear.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests