Reviewer’s report

Title: A longitudinal study of gene expression in healthy individuals

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Reviewer: martin petrek

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Karlovich et al describe here the gene expression profiles in healthy men and women in whole blood and their variability over period of 6 months. They used two approaches (qRT-PCR and microarray) to assess the differences in expression profiles in subgroups according gender, age. This is an original article which addressed an important question in the field of gene expression, but there are several limitations with this study, which are addressed below.

Major compulsory revisions

1) The authors combined in this paper the Result and discussion. According opinion of this reviewer, in fact there is no discussion on the presented data. The authors acknowledge David Trollinger of Source Precision Medicine for discussions regarding the genes chosen for qRT-PCR, however they do not explain their choice to the readers. I also miss the explanation about the selection of cut-off for age. There is no discussion about the data and their practical importance. Their conclusions are not based on the data obtained and are to vague, or let me say general (e.g. Page 19- „These parameters can be used to estimate the number of subjects needed to observe significant differences from normal gene expression in clinical studies.”)

2) It seems that no ethics committee permitted this study; the subjects did not sign the informed content. This is a VERY MAJOR FLAW!

3) The authors performed the microarray analysis and qRT-PCR separately; they did not combine the results. It seems that two different studies were performed and just joined together.

4) The study is presented as an analysis of 80 subjects (they called this “main” study), however, one ¼ (25%) were analysed in fact. Especially for qRT-PCR is the number very low- qRT-PCR was performed in only 22 subjects (subgroups: 8 young females, 7 older females, 5 young males, 8 older males). There is a very low overlap of samples used for both techniques (“Samples from eight subjects were analyzed on both qRT-PCR and microarray”). Especially, when they used such little RNA per qRT-PCR reaction (10 ng)? We recommend to analyse the expression profile by qRT-PCR in all enrolled subjects.

5) RNA quality was assessed on an Agilent Bioanalyzer 2100. Why the authors did not quantify the RNA samples by this technique, they wrote that “samples were quantified using ribogreen, a nucleic acid stain”.

6) Expertise from statistician is needed.
7) All qRT-PCR data are presented as raw Ct. However, the intra- and inter-assay variability may have influence on the presented data. Did the authors use any calibrator? How was the amplification efficiency in all genes? I recommend presenting the data as ratio to reference gene, not as Ct raw data. How significant was the variation between several lots of chemicals (page 19-“A measurable bias was introduced when samples from the present study were processed and analyzed with different reagent lots over time in sequential batches (data not shown).“)? The authors mentioned this without showing the data (please make additional file from this).

Minor essential revisions

1) I would recommend to change the title to” …healthy non-smokers”. In the subject description it should be defined more precisely if there were some ex-smokers enrolled into this study.

2) Page 1-Abstract: Results about the patients with cancer and anemia should not be main-results from the study. Two patients are not enough for conclusions.

3) Please give more information about how much RNA was obtained, how many ml of blood were taken to PAXgene tube.

4) Page 12-„shifted to the left end of the normal laboratory range“. Please define the left end.

5) Page 7. “……with minor modifications”. However, the used modification is not mentioned.

6) Selected genes were measured by qRT-PCR at four time points (Baseline, Day 28, Day 90 and Day 180), however they obtained samples from „five scheduled visits on Days 1, 14, 28, 90, and 180 per protocol.“

7) Additional file 3-The data are given in Fold Change (which group was set to 1?)

8) Additional file 2- Norm = normalized values, it is not mention against which gene is normalised

9) Table 2 - Hematology values of all subjects over all time points-it would be more interested to see changes in individuals over six months

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

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests. MP