

## **Author's response to reviews**

**Title:** Sex hormones and gene expression signature in peripheral blood from postmenopausal women - the NOWAC postgenome study

### **Authors:**

Marit Waaseth ([marit.waaseth@uit.no](mailto:marit.waaseth@uit.no))  
Karina S. Olsen ([karina.standahl.olsen@uit.no](mailto:karina.standahl.olsen@uit.no))  
Charlotta Rylander ([charlotta.rylander@uit.no](mailto:charlotta.rylander@uit.no))  
Eiliv Lund ([eiliv.lund@uit.no](mailto:eiliv.lund@uit.no))  
Vanessa Dumeaux ([vanessa.dumeaux@uit.no](mailto:vanessa.dumeaux@uit.no))

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## Responses to reviewer's report

### Minor concerns:

In the background section ...T is not defined references 10 and 11.

- *“T” is now defined at first appearance.*

There is no consistency in the RNA isolation because it states that 328 and 39 samples were excluded due to lack of quality. Since we have these data then we have 289 samples for microarray RNA analysis and the authors final expression matrix comprised 285 samples (page 6 and 7). Please clarify this.

- *We agree that this information may be confusing, but the exclusion of samples continues in the “Data analysis”-section. Samples where less than 40% of the probes showed a signal-to-noise ratio (S/N)  $\geq 3$  were excluded, as well as one sample misclassified as postmenopausal. We have now specified the number of samples excluded due to low S/N (n=3).*

P4 is not defined (page 7)

- *“P4” was defined at page 6 (first appearance)*

Figure 4 is cited not in properly in order.

- *In our revised manuscript the figures now appear in consecutive order in the text.*

Typo mistakes page 14 discordant

- *We have corrected the spelling error*

### Major concerns:

The title is too broad not according to the conclusion of E2/P novel gene expression signature.

- *We acknowledge that the title might seem broad compared with the conclusion, although the study's objective is broad in itself. We have now slightly rephrased both title and conclusion to clarify.*

Figure 1 is never listed in the article.

- *We have included reference to figure 1 in the text*

Figure 2 is not properly cited and the figure legend is poor not self explanatory. In the case of E2 tx alone even they stated not enriched clusters, is convenient to described the 10 genes associated with E2 versus slone medication.

- *We agree that the figure legend is poor and have provided a rephrased figure legend for Figure 2. For the remaining of the comment we are a bit uncertain what it points to. Figure 2 was originally cited on page 9, but is now also mentioned on pages 11 and 13.*
- *Regarding describing the 10 genes associated with E2 alone although they did not predict any gene networks: We have provided some information on a few genes (from both estradiol gene sets) that are interesting in relation to hormones. In other respects,*

*describing single, unconnected genes gives little meaning since genes hardly ever function alone.*

Which is the role of tibolone in these patients being only 2 analyzed?

- *Tibolone is an HT, a synthetic steroid, and do not contain estradiol. It should therefore be analyzed as a separate HT category. There were 10 users altogether, but only 2 of these did not use any other medication. Since two samples constitute a very weak basis for conclusions regarding tibolone and gene expression, we included genes that were also present when all 10 users were included in the analysis.*

More discussion about the physiological implications of these findings.

- *We have included references to physiology for a few genes. However, considering the inconclusive results from the functional enrichment analysis and that the reproducibility remains to be tested, we think it would be inappropriate to hypothesize further regarding physiological implications of our results at present.*

Questions: These new gene expression signature. Which are the explanations of the genes that are crosslinked between the group of HT versus no medication E2/P and E2 systemic alone vs no medication.

- *We acknowledge a want of discussion regarding these genes and have elaborated on the subject in both the results section and the discussion.*

## **Editorial Requests:**

Copyedit

Please get your manuscript copy edited. Perhaps a colleague, preferably a native English speaker, could look it over for you. Alternatively, we can supply the contact details of a professional copy editing service.

- *The manuscript has now been copy edited*

Ethics/consent

Please can you state whether your research conformed to the Helsinki Declaration (<http://www.wma.net/e/policy/b3.htm>), and to local legislation. Please add a paragraph in your methods section stating this, and name the ethics committee which approved the research. Please also indicate whether patients gave informed consent to participate in the study.

- *We have added the requested information*

Questionnaire - Please provide a copy of your questionnaire as an additional file.

- *We have provided a translated copy of our questionnaire*