Reviewer's report

Title: The ceramide [NP]/[NS] ratio in the stratum corneum is a potential marker for skin properties and epidermal differentiation

Version: 0 Date: 03 Jun 2019

Reviewer: Kateřina Vávrová

Reviewer's report:

The authors analyzed ratios of various skin ceramide subclasses in healthy human skin, lesional and nonlesional skin of patients with atopic dermatitis, in patients with psoriasis, and in cultured keratinocytes. They report that the ratio of phytosphingosine-based Cer NP to sphingosine-based Cer NS correlates with TEWL, capacitance and skin appearance, and varies between diseased and healthy skin. This ratio also increased with differentiation of healthy keratinocytes. These findings are interesting, methods are adequate and data support most of the conclusions. I have the following points to consider:

1. The Cer NP/NS is suggested to be a marker for healthy/diseased skin. Did you try to predict the skin health of blinded skin samples and what was the outcome? If not, your conclusions should be attenuated.

2. There are also correlations between TEWL and other Cer ratios (Table 1). If there is a link to DEGS2 as suggested, one may expect a strong correlation of the ratio of all phytosphingosine Cer combined (NP, AP, EOP) to all sphingosine Cer (NS, AS, EOS) and the studied parameters - please add and discuss.

3. What is the possible rationale behind the increased Cer NP/NS ratio in healthy skin compared to diseased skin? Is there any apparent benefit of increased Cer NP over Cer NS in the SC lipids, for example in terms of decreased permeability or water loss? The discussed Cer NP production by DEGS2 may be an answer how but not why this happens. Should this ratio be a marker with potential clinical relevance, the mechanisms underlying these changes should be understood.

4. Did you see any changes in the overall chain lengths in Cer NP and Cer NS? In atopic dermatitis, increased shorter-chain Cer NS (mostly with C16 acyl) has been reported, is it possible that Cer NP/short Cer NS may be an even better marker?

5. Did you see any change of the Cer NP/NS ratio with age in healthy volunteers?

6. The discussion section is hard to read, please split it into paragraphs and avoid repeating results.

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?
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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