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SASPase regulates stratum corneum hydration through profilaggrin-to-filaggrin processing

Takeshi Matsui, Kenichi Miyamoto, Akiharu Kubo, Hiroshi Kawasaki, Tamotsu Ebihara, Kazuya Hata, Shinya Tanahashi, Shizuko Ichinose, Issei Imoto, Johji Inazawa, Jun Kudoh and Masayuki Amagai

Corresponding author: Takeshi Matsui, Tokyo Medical and Dental University

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 29 October 2010

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now received the three enclosed reports on it. You will see that they find the topic of your manuscript potentially interesting but they feel that the data need to be strengthened in some areas. This should be addressed in a major revision.

As you will see, all reviewers raise the point that human samples should be further investigated. In our opinion, of particular importance is here the investigation of profilaggrin processing in AD patients with SASPase mutations. In addition, we feel that the analysis of NMF levels in the knockout mice would be critical to strengthen the manuscript as pointed out by reviewer #2.

Given the balance of these opinions, we feel that we can consider a revision of your manuscript if you can address the technical concerns that have been raised within our space and time constraints. Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions, unless arranged otherwise with the editor.

I look forward to seeing a revised form of your manuscript as soon as possible.

Yours sincerely,

Editor
EMBO Molecular Medicine
REFEREE REPORTS:

Referee #1 Comments on Novelty/Model system:

This is an interesting manuscript of potential importance. The major weakness is the authors need to provide more information on their studies in humans. If the results in humans agree with the results presented in the KO mice this would be a notable advance.

Referee #1:

Major issues
1) The results described in the present study should be compared to the results seen by other investigators using the Flaky tail mouse, which also has a defect in filaggrin metabolism. For example, a very large decrease in SC hydration is not observed in the flaky tail mouse but is seen in the SASPase deficient mouse. Additionally, the marked increase in the number of layers of the SC is not observed on the back of flaky tail mice. This suggests that the buildup of certain filaggrin degradation products may lead to changes rather than the absence of end products. Alternatively, SASPase may be involved in the metabolism of other proteins leading to some of the observed changes. These issues should be discussed in the discussion section of the manuscript.
2) The human data while interesting requires additional information. Specifically, do the patients with these polymorphisms have abnormal filaggrin? Do these patients have skin abnormalities in areas that do not have atopic dermatitis? Demonstrating abnormalities similar to what is observed in the KO mice is very important.

Minor issues
1) Figure 3A- The increase in TEWL is not significant in the +/- mice. However, the number studied is only n=5. This number should be increased.
2) Figure 4- The negative data shown in this figure can be moved to supplementary figures. The positive data shown in supplementary figure 2 could be shown as a figure in the manuscript.
3) Figure 5C- Why is the quantity of profilaggrin decreased in the +/- mice?

Referee #2 Comments on Novelty/Model system:

See comments below

Referee #2:

The article of Matsui et al. 'SASPase regulates stratum corneum hydration through profilaggrin to filaggrin processing' builds further on earlier work by the group on the SASPase protease. In a previous paper they described the development of SASPase deficient mice (Matsui et al., 2006), and performed some initial characterization of these KO mice. In the current paper the authors report convincingly that SASPase is involved in the processing of profilaggrin to the filaggrin monomeric subunit. This is an important finding explaining the dry skin observed in the SASPase KO mice. In addition, they show that recombinant SASPase is able to directly cleave the linker peptide of recombinant profilaggrin. Finally, the authors identified a number of missense mutations in the SASPase gene in samples derived from atopic dermatitis patients and from normal individuals. Among these, some mutants were affected in their enzymatic activity. Further follow-up work is needed, as recognized by the authors, to unravel the role of SASPase deficiency in susceptibility to develop atopic dermatitis or related skin inflammatory diseases. This is an interesting paper, with clinical relevance to skin inflammatory diseases, adding to our knowledge of profilaggrin processing. Importantly, the authors provide genetic evidence for the involvement of SASPase in profilaggrin processing. However, some remarks should be made to further improve the paper.

Scientific remarks
1. Fig 4 can be omitted, it is a repetition of Fig 8 in Matsui et al, 2006 (just the genetic background is different in the present manuscript), or at least shift to supplementary info.
2. Fig 5: How do the authors explain that the altered (pro)filaggrin localization in the SASPase-/- mice is not present in the filaggrin stainings presented in Matsui et al, 2006? Could this be explained by the different genetic background? How consistent is this observations throughout different skin sections? For example, in Fig 2A and B you can also observe some keratinohyalin granules in the lower part of the SG in WT mice.

3. Page 9, line 213: '...other proteins were also decreased (Figure 5C, left)'. How did the authors check that similar amounts of total protein were loaded?

4. Fig 5: This figure nicely shows that the profilaggrin processing is affected in SASPase-/- mice. The morphological analysis suggests that the keratin aggregation activity of the filaggrin dimers and trimers is probably not affected, as indicated by the normal appearance of the SG. One essential important question remaining is whether these filaggrin fragments are still degraded to NMFs or not. It would be an enormous asset for the impact of this paper to analyze whether NMF levels (UCA, PCA) are affected in the SASPase-/- mice.

5. Fig 7: 1) Fig 7C is not convincing. It does not make sense to look in the total bacterial lysate. The fact that you do not observe low levels of hSASP14 could be due to many reasons (as illustrated by the results obtained with the V187I mutant in Fig 7C versus Fig 7F). Therefore Fig 7C should be omitted. 2) I don't think it has been sufficiently proven that the V187I affects the proteolytic activity of the SASPase as such (Fig 7G and page 13, line 306-307). The result could also be explained by the fact that there is less active hSASP14 form in the reaction mixture. Due to the lack of similar amounts of hSASP14 for the different mutants, this experiment is not conclusive and should be omitted (also rephrase this section in the abstract and in the discussion at page 16, line 374). The authors should compare similar amounts of the active hSASP14 form.

6. In case the authors would have skin samples of the mutant patients available, it would be interesting to check profilaggrin processing in these samples.

Editorial remarks
1. Page 4, line 94: The authors should discriminate the tandem repeat number between mouse and men.
2. Page 5, line 122: It is better to stick to one name for the same protease, namely SASPase, I would not use Taps in the sentence 'Recently, aberrant Taps...'  
3. Page 6, lines 146-150: Mention in one sentence that SASPase can not be detected in SASPase deficient mice both in western blotting and IHC.
4. Page 10, line 233: '...due to excess degradation...' in stead of '...due excess degradation...'.
5. Fig 6E: This very comprehensive scheme should be omitted from Fig 6 and should be used as a separate, concluding figure of the manuscript.

Referee #3 Comments on Novelty/Model system:

The evidence for the role of SASPase mutations in human atopic dermatitis is low.

Referee #3:

The paper describes a SASPase mouse model and a possible role of SASPase in human atopic dermatitis. However the proof of the pathogenic relevance of SASPase sequence variants in atopic dermatitis is difficult to achieve.

Comments:
1. Page 7, line 183 and the Figure 2C-D and the supplementary figure 2: Stratum corneum layers in SASPase +/- and -/- mice: there is a conflict between the sentence on page 7, line 183 and the Figure 2C-D and the supplementary figure 2. Both figures show increased number of stratum corneum layers in the SASPase +/- mouse, whereas the sentence claims the opposite.
2. Figure 6E is not mentioned in the manuscript text.
3. The title of the Supplemental Table II "Nonsense mutations...." is inexact. These are not nonsense mutations.
4. The evidence for the implication of the SASPase sequence variants in human AD is relatively low. The number of controls is too small. Moreover, one might know whether the controls have dry skin or not. In the case of complex disorders, like AD, it is difficult to prove the direct role of the sequence variant in the pathogenesis. Even if both, I186 and V187, are very close to the autoprocessing site, they seem to have different effects. It is not clear to me whether the reaction conditions used in the experiments are comparable to the physiological situation in the skin (Figure 7C, E, F).

5. In the case of V187I, patients are heterozygous for the wild type allele, but the mutated seem to have a residual functional activity. How much would the enzymatic activity be?

6. Could the authors show abnormal profilaggrin / filaggrin profile in patients with V187I, as they show in /-/- mice (Figure 5C)

7. How can the authors explain that V243A found in controls, lacks autoprocessing activity. Did the individual have dry skin?

8. Could variants with autoprocessing activity have reduced enzymatic activity because of loss of other functions?

9. Did the authors analyse the gene in patients with ichthyosis vulgaris?

1st Revision - Authors’ Response 28 January 2011

We are grateful for the editor’s and the reviewers’ valuable comments and the opportunity to resubmit our manuscript. We have seriously considered each reviewer’s remarks and attached our point-by-point answers.

Editor

In our opinion, of particular importance is here the investigation of profilaggrin processing in AD patients with SASPase mutations. In addition, we feel that the analysis of NMF levels in the knockout mice would be critical to strengthen the manuscript as pointed out by reviewer #2.

Answer: The reviewers identified two major issues. The first referred to the levels of NMF in SASPase knockout mice. We carried out further analyses and found that NMF levels were not altered in SASPase knockout mice, indicating that the accumulation of aberrantly processed profilaggrin and the marked decrease in mature filaggrin had an impact on the texture and hydration of the stratum corneum (SC).

The second issue involved the investigation of profilaggrin processing in human atopic dermatitis (AD) patients with SASPase mutations. We attempted to carry out further analyses to address this issue. We optimized a protocol to detect profilaggrin processing in human SC samples, and realized that we needed to tape strip up to 40 times to obtain the lower layers of profilaggrin-containing SC. This caused severe pain in tested individuals, so we concluded that the detection of profilaggrin processing in human SC is not practical. We then attempted to recruit AD patients and normal individuals with the mutations to measure TEWL and SC hydration, which are non-invasive tests. However, we had difficulty recruiting participants as these tests were not included in the initial consent. We recruited one non-AD control with mutations and three normal individuals without mutations and obtained preliminary results. Although we described some of the results in the revised manuscript, they are non-conclusive due to the small numbers tested. We agree that the human study is important, but it is beyond the scope of our manuscript.

Referee #1

Comment #1:

The results described in the present study should be compared to the results seen by other investigators using the Flaky tail mouse, which also has a defect in filaggrin metabolism. For example, a very large decrease in SC hydration is not observed in the flaky tail mouse but is seen in the SASPase deficient mouse. Additionally, the marked increase in the number of layers of the SC is not observed on the back of flaky tail mice. This suggests that the build-up of certain filaggrin degradation products may lead to changes rather than the absence of end products. Alternatively,
**SASPase may be involved in the metabolism of other proteins leading to some of the observed changes. These issues should be discussed in the discussion section of the manuscript.**

Answer: We agree with the reviewer’s comments. We have taken into account the phenotype of flaky tail mice and suggested that accumulation of aberrantly processed profilaggrin has an impact on the texture and hydration of the SC. We also described the possibility that other substrates of SASPase are involved in decreased SC hydration. We have addressed these issues in the Discussion section of the revised manuscript: P16, L. 370-378 and P16, L. 388 - P17, L. 395.

**Comment #2:**

*The human data while interesting requires additional information. Specifically, do the patients with these polymorphisms have abnormal filaggrin? Do these patients have skin abnormalities in areas that do not have atopic dermatitis? Demonstrating abnormalities similar to what is observed in the KO mice is very important.*

Answer: To address the reviewer’s remarks, we optimized a protocol to detect the profilaggrin processing pattern in human SC samples. These data indicate that to obtain the typical processing pattern from the lower layers of profilaggrin-containing SC (lower SC), we need to tape strip participants up to 40 times. As this would cause severe pain in the test individuals, we conclude that the detection of profilaggrin processing in human SC is not practical.

We then attempted to recruit AD patients and normal individuals with the mutations to measure TEWL and SC hydration, which are non-invasive tests. However, as these tests were not included in the initial consent, we were only able to recruit one ‘non-AD’ individual with heterogenic mutation (V243A+/+) and three normal individuals without mutations (controls). We obtained the preliminary results shown in Supplementary Figure 3. The appearance of the skin surface, TEWL, and SC hydration of theV243A/+ individual were relatively normal compared with the three controls. These results were unfortunately non-conclusive due to the small numbers tested. We agree that the human study is very important but it is beyond the scope of our manuscript. Meaningful data can only be obtained from a future large scale cohort analysis.

We addressed these issues with a Figure and some additions to the text of the manuscript: Supplementary Figure 3; P. 14, L. 330-340.

**Minor issues**

1. **Figure 3A** - The increase in TEWL is not significant in the SASP+/+ mice. However, the number studied is only n=5. This number should be increased.

Answer: We agreed with the reviewer’s comments and increased the numbers of SASP+/+ and SASP−/− mice to n = 7 and n = 11, respectively. We measured TEWL and SC hydration again and obtained the same results as were previously recorded. We changed Figure 3 and several sentences in the manuscript accordingly: Figure 3; P. 8, L. 190-195, P. 20, L. 463 and P24, L. 572-573.

2. **Figure 4** - The negative data shown in this figure can be moved to supplementary figures. The positive data shown in supplementary figure 2 could be shown as a figure in the manuscript.

Answer: We have changed ‘Supplementary Figure 2’ into ‘Figure 2H’ and ‘Figure 4’ into ‘Supplementary Figure 2’.

3. **Figure 5C** - Why is the quantity of profilaggrin decreased in the SASP−/− mice?
Answer: The Bradford method was used to quantify the protein concentration of total lysates and calculate the amount loaded of each sample. The weak intensity of profilaggrin in SASP+/− epidermis was probably due to the accumulation of large amounts of aberrantly processed profilaggrin, which resulted in correspondingly lower levels of profilaggrin. In accordance with these remarks, we changed the sentences that referred to Figure 4C in the revised manuscript: P. 9, L. 209-214, P. 20, L. 472-473, P. 25 L. 586 and L. 592-594.

Referee #2

Comment #1:

Fig 4 can be omitted, it is a repetition of Fig 8 in Matsui et al, 2006 (just the genetic background is different in the present manuscript), or at least shift to supplementary info.

Answer: In accordance with this suggestion, we changed Figure 4 to Supplementary Figure 2.

Comment #2:

Fig 5: How do the authors explain that the altered (pro)filaggrin localization in the SASPase−/− mice is not present in the filaggrin stainings presented in Matsui et al, 2006? Could this be explained by the different genetic background? How consistent is this observation throughout different skin sections? For example, in Fig 2A and B you can also observe some keratinohyalin granules in the lower part of the SG in WT mice.

Answer: The indistinguishable filaggrin staining in the previous paper may be due to the thin epidermis of the C57BL/6J mouse back skin and the presence of hair, compared with Hos:HR-1 hairless background used in this study. We addressed this issue by adding the following sentences to the text of the manuscript: P. 15, L. 347-352. The reproducibility of our data was confirmed by Figures 2 and 4, which were derived from different littermates.

Keratohyalin granules were found in both SASP+/+ and SASP−/− hairless mice (Figure 2A, B, C, D). We could not identify any differences in the numbers and sizes of the keratohyalin granules in the SGs of SASP+/+ and SASP−/− hairless mice by electron microscopy. Profilaggrin in keratohyalin granules is presumably not processed by SASPase. Thus, it is likely that keratohyalin granules exist normally in SASP−/− mice.

Comment #3:

Page 9, line 213: ‘…other proteins were also decreased (Figure 5C, left)’. How did the authors check that similar amounts of total protein were loaded?

Answer: The Bradford method was used to quantify the protein concentration of total lysates and calculate the amount of each loaded sample. Please refer to the answer to Reviewer #1, Minor issue #3 for more details.

Comment #4:

Fig 5: This figure nicely shows that the profilaggrin processing is affected in SASPase−/− mice. The morphological analysis suggests that the keratin aggregation activity of the filaggrin dimers and trimers is probably not affected, as indicated by the normal appearance of the SG. One essential important question remaining is whether these filaggrin fragments are still degraded to NMFs or not. It would be an enormous asset for the impact of this paper to analyze whether NMF levels (UCA, PCA) are affected in the SASPase−/− mice.
Answer: We agreed with the reviewer’s suggestions and analyzed whether NMF levels were affected in SASP+/− mice. We found no difference between the free amino acid composition of SASP+/− and SASP−/− mice, which suggests that NMFs are normal in SASP−/− hairless mice. Unfortunately, because of time constraints, we could not perform UCA and PCA analyses. We added a new Figure and some sentences to the revised manuscript: Figure 6; P. 2, L. 53-54, P. 11, L. 261-268, P. 15, L. 356-363, P. 16, L. 370-378, P. 20, L. 475-481, P. 26, L. 617-622.

Comment #5:

Fig 7: 1) Fig 7C is not convincing. It does not make sense to look in the total bacterial lysate. The fact that you do not observe low levels of hSASP14 could be due to many reasons (as illustrated by the results obtained with the V187I mutant in Fig 7C versus Fig 7F). Therefore, Fig 7C should be omitted.

Answer: We omitted Figure 7B and C as both were data derived from the analyses of total bacterial lysates. We deleted several sentences related to Figure 7B and C.

Comment #6:

I don't think it has been sufficiently proven that the V187I affects the proteolytic activity of the SASPase as such (Fig 7G and page 13, line 306-307). The result could also be explained by the fact that there is less active hSASP14 form in the reaction mixture. Due to the lack of similar amounts of hSASP14 for the different mutants, this experiment is not conclusive and should be omitted (also rephrase this section in the abstract and in the discussion at page 16, line 374). The authors should compare similar amounts of the active hSASP14 form.

Answer: As shown in Figure 7A, V187I is located outside of the protease domain. Thus, the auto processed product (hSASP14) of GST-hSASP28(V187I) is the same as wild type. As shown in the reaction curve of Figure 8E, incubation of GST-hSASP28(V187I) at pH 6.0 revealed that the V187I mutation affected the initial auto processing reaction (0–30min; Figure 8D, E). Later (30–45min), the processing reaction seemed unaffected by an increased amount of hSASP14. These data indicate that the first 0–30min reflect the difference in activity between WT and V187I. These issues are described in detail in the Results and Discussion sections of the revised manuscript: Figure 8D and E; P. 13, L. 313-321, P.21, L. 489-490 and P. 28, L. 660-664.

We also deleted ‘and profilaggrin linker cleavage activity’ from sentence: P.17, L. 402-404.

Comment #7:

In case the authors would have skin samples of the mutant patients available, it would be interesting to check profilaggrin processing in these samples.

Answer: We agree with the reviewer’s comment; however, checking profilaggrin processing in human samples is difficult due to problems associated with sample collection. For a discussion of these issues, please refer to the response to Reviewer #1 Comment #2.

Editorial remarks

1. Page 4, line 94: The authors should discriminate the tandem repeat number between mouse and men.

Answer: We described the human repeat number of profilaggrin in the revised manuscript: P. 4, L. 93.
2. Page 5, line 122: It is better to stick to one name for the same protease, namely SASPase, I would not use Taps in the sentence 'Recently, aberrant Taps...'

   Answer: We changed ‘Taps’ to ‘SASPase’: P. 5, L. 121.

3. Page 6, lines 146-150: Mention in one sentence that SASPase can not be detected in SASPase deficient mice both in western blotting and IHC.

   Answer: In the revised manuscript, we mentioned that SASPase cannot be detected in SASPase deficient mice by western blotting or IHC: P. 6, L. 149-151.

4. Page 10, line 233: ‘...due to excess degradation...’ in stead of ‘...due excess degradation...’.

   Answer: We corrected the sentence: P. 10, L. 234.

5. “Fig 6E: This very comprehensive scheme should be omitted from Fig 6 and should be used as a separate, concluding figure of the manuscript.”

   Answer: We changed Figure 6E to Figure 7. Figure 7 is a concluding separate figure, which we have described in the Results section of the revised manuscript: P. 11, L. 270 – P. 12, L. 279 and P. 26, L. 624 – P. 27 L. 636.

Referee #3

Comments #1:

Page 7, line 183 and the Figure 2C-D and the supplementary figure 2: Stratum corneum layers in SASPase +/+ and -/- mice: there is a conflict between the sentence on page 7, line 183 and the Figure 2C-D and the supplementary figure 2. Both figures show increased number of stratum corneum layers in the SASPase -/- mouse, whereas the sentence claims the opposite.

Answer: We corrected the sentence: P. 8, L. 183-185.

Comments #2:

Figure 6E is not mentioned in the manuscript text.

Answer: We changed Figure 6E to Figure 7 and described it in the Results section: P. 11, L. 270 – P. 12, L. 279 and P. 26, L. 624 – P. 27 L. 636.

Comments #3:

The title of the Supplemental Table II "Nonsense mutations...." is inexact. These are not nonsense mutations.

Answer: We changed the title of Supplemental Table II to “Silent mutations of SASPase in AD patients.”
Comments #4:

The evidence for the implication of the SASPase sequence variants in human AD is relatively low. The number of controls is too small. Moreover, one might know whether the controls have dry skin or not. In the case of complex disorders, like AD, it is difficult to prove the direct role of the sequence variant in the pathogenesis.

Answer: We agreed with reviewer’s comments. We recognized that our cohort was too small to analyze the statistical significance of SASPase mutation. In addition, it is possible that our control had dry skin because we corrected AD and non-AD (control) individuals according to the criteria described in the previous report outlined below:

‘Blood samples were obtained from 24 Japanese AD patients, who have been suffering from severe, itchy, chronically relapsing, inflammatory skin condition for a long time, and observation of total IgE amount incensement. The diagnosis of atopic dermatitis was made using the AD diagnostic criteria by Japanese Dermatological Association. Among these 24 Japanese AD patients, there is no IV patient who shows palmar hyperlinearity and typical scaling at arms and legs.’


Therefore, we could not discriminate dry skin in our cohort. We added a sentence to the text of the revised manuscript discussing this issue: P. 14, L. 331-333.

We are in complete agreement with the comment addressing the difficulty of understanding the pathology of AD by a single sequence variant. We know it is necessary to perform a large scale cohort analysis to clarify the clinicopathological significance of the SASPase mutation. Thus, we included a discussion of these issues in the revised manuscript: P. 14, L. 330-340.

Comments #5:

Even if both, I186 and V187, are very close to the auto processing site, they seem to have different effects. It is not clear to me whether the reaction conditions used in the experiments are comparable to the physiological situation in the skin (Figure 7C, E, F).

Answer: According to our previous report (Matsui et al., J. Biol. Chem. 281:27512-27525, 2006), under physiological conditions (150 mM NaCl and pH 7.5), protease activity is substantially reduced. Therefore, to compare the difference in activity within 45 min in vitro, we had to examine protease activity under a high NaCl concentration (0.7 M) and the optimal pH (pH 6.0). We added this point in the following sentence in the revised manuscript: P. 13, L. 306-307.

In this report, we identified a differential effect on the activity by I186T and V187I of ‘human SASPase’. Previously, we developed a fluorogenic peptide consisting of eight amino acids of ‘mouse SASPase’ (Matsui et al., 2006).

Furthermore, we recently confirmed that a point mutation of the corresponding P4 position of mouse SASPase “L185” substantially reduced its auto processing activity (data not shown). These data suggest that eight amino acids are minimal for substrate recognition, and that P4 positions ‘V187 in human’ and ‘L185 in mouse’ are important for their auto processing activities.

Comments #6:

In the case of V187I, patients are heterozygous for the wild type allele, but the mutated seem to have a residual functional activity. How much would the enzymatic activity be?

Answer: We semiquantified the auto processing product, hSASP14 and showed the time course of auto processing activity in Figure 8E. It revealed that the initial velocity of the auto processing reaction was not linear, suggesting that auto processed hSASP14 is also involved in the later processing reaction. Thus, it is suggested that the first 0–30 min (300 mM NaCl) reflect the difference in activity between WT and V187I. We estimated that the activity of GST-hSASP28(V187I) was reduced 5.6-fold. We added an extra Figure and the following sentences to our revised manuscript: Figure 8E; P. 13, L. 313-321, P.21, L. 489-490 and P. 28, L. 660-664.

Comments #7:

Could the authors show abnormal profilaggrin / filaggrin profile in patients with V187I, as they show in -/- mice (Figure 5C).

Answer: This is a very important comment that was also mentioned by Reviewer #1 (Comment #2) and Reviewer #2 (Comment #7). However, identifying an abnormal profilaggrin/filaggrin profile in human samples is difficult due to problems associated with sample collection. For a discussion of these issues please refer to the response to Reviewer #1 Comment #2.

Comments #8:

How can the authors explain that V243A found in controls, lacks auto processing activity. Did the individual have dry skin?

Answer: This is a very important comment. As discussed in our reply to this reviewer’s Comment #4, we could not discriminate dry skin in our cohort because of the criteria we used to correct AD and non-AD (control) individuals. For a full description of this issue please see Reviewer 3 Comment #4 and sentence: P. 14, L. 331-333 in the revised manuscript.

Comments #9:

Could variants with auto processing activity have reduced enzymatic activity because of loss of other functions?

Answer: SASPase (V187I) has reduced auto processing activity in vitro. For a more detailed discussion, please see the answers to Comments #5 and #6.

Comments #10:

Did the authors analyse the gene in patients with ichthyosis vulgaris?

Answer: We are now planning to carry out a large scale cohort analysis of AD and ichthyosis vulgaris patients.
Other corrected points:
1. We added ‘linker’ to sentence: P. 5, L. 115.
2. We corrected the name Hos:HR-1: P. 6, L. 155.
3. We added references related to the NMFs and skin moisturization: P. 11, L. 262-263, P. 15, L. 345-346 and P. 33, L. 818-819.
5. We transferred “Preparation of mice SC urea-extracts” and “cDNA cloning and recombinant protein expression” in Materials and methods section to Supplemental information.

2nd Editorial Decision 21 February 2011

Thank you for the submission of your revised manuscript "SASPase regulates stratum corneum hydration through profilaggrin-to-filaggrin processing" to EMBO Molecular Medicine. We have now received the enclosed reports from the referees that were asked to re-assess it. As you will see the reviewers are now globally supportive and I am pleased to inform you that we will be able to accept your manuscript pending the following final amendments:

- Reviewer #1 points out that the discussion of SC hydration should be extended to include other factors besides profilaggrin/filaggrin processing, which could contribute to SC hydration in the mutant mice.
- We also agree with Reviewer #2 that it would improve the text if Fig 7 would be presented at the end of the manuscript.

Please submit your revised manuscript within two weeks. I look forward to seeing a revised form of your manuscript as soon as possible.

Yours sincerely,
Editor
EMBO Molecular Medicine

REFEREE REPORTS:

Referee #1 Comments on Novelty/Model system:

This revised manuscript has addressed most of my concerns. It would have been nice if they could have provided data on the effect of the mutations in SASPase on skin structure and function in humans but I can understand that this is a difficult task beyond the scope of the present manuscript. The only area they need to improve is their discussion of stratum corneum hydration (see comments to authors below)

Referee #1:

The discussion of stratum corneum hydration needs to be improved. Specifically, the authors need to note that a variety of compounds can play important roles in maintaining stratum corneum hydration. For example, studies have shown that glycerol plays an important role in stratum corneum hydration. It is possible that the decreased stratum corneum hydration in SASPase deficient mice is related to alterations in the levels of these other compounds. I think the authors need to expand their discussion to include possibilities in addition to filaggrin metabolism possible contributing to the decrease in hydration in the SASPase deficient mice.

Referee #2 Comments on Novelty/Model system:

Technical: experiments and interpretations were done adequately. The authors used a multitude of
techniques to proof their conclusions.

Novelty: The authors identified a new profilaggrin processing enzyme with potential medical relevance for understanding dry skin and possibly atopic dermatitis.

Medical impact: unfortunately the authors could, for the time being, not address the situation in patients. However, it is quite plausible that a subset of AD patients will have SASPase mutations.

Referee #2:

The authors addressed adequately the reviewers comments. Unfortunately they could not address, for the time being, the contribution of SASPase mutations in patient populations.

One small remark: I would shift Fig 7 to Fig 8 and vice versa because Fig 7 is a summarizing figure of SASPase function.

2nd Revision - Authors' Response 11 March 2011

We are grateful for the editor’s and the reviewers’ valuable comments on our manuscript. We have considered each reviewer’s remarks and attached our point-by-point responses.

Referee #1

Comment #1:

The discussion of stratum corneum hydration needs to be improved. Specifically, the authors need to note that a variety of compounds can play important roles in maintaining stratum corneum hydration. For example, studies have shown that glycerol plays an important role in stratum corneum hydration. It is possible that the decreased stratum corneum hydration in SASPase deficient mice is related to alterations in the levels of these other compounds. I think the authors need to expand their discussion to include possibilities in addition to filaggrin metabolism possible contributing to the decrease in hydration in the SASPase deficient mice.

Answer: We fully agree with the reviewer’s comments. Therefore, we have expanded our discussion to include other possibilities in addition to filaggrin metabolism along with appropriate citations:


Referee #2

Comment #1:

I would shift Fig 7 to Fig 8 and vice versa because Fig 7 is a summarizing figure of SASPase function.

Answer: In accordance with this suggestion, we have switched the order of Figures 7 and 8. Furthermore, in Figure 8, we have replaced ‘NMFs’ with ‘Free amino acids’ and changed the result and legend to better describe our findings.


Other corrected points:

1. We have changed the affiliation of the 8th author: P. 1, L. 23–24.

2. We have changed the word of Abstract: P. 2, L. 62.

3. We have changed some references in the Introduction: P. 4, L. 101–102.