Author’s response to reviews

Title: Langerhans cells in hypospadias: An analysis of Langerin (CD207) and HLA-DR on epidermal sheets and full thickness skin sections

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Author’s response to reviews:

Dear Mrs. Cryer! Dear Editor!

We appreciate the fair and constructive comments the reviewers made and your comment below on how to clearly highlight potential limitations to our study. By working on the manuscript, we feel that it has improved in clarity and quality.

We addressed your comments below, the respective changes to the manuscript are in blue.

We hope you can share our view on having replied satisfactorily and consider our manuscript for publication in your distinguished journal.

Sincerely!

Bernhard Haid on behalf of the authors

Editor comments:
The reviewers have highlighted a number of points which feel you may be able to address, once of
which being fully addressing the full limitations of your work. When discussing your research limitations, do not just provide the list of shortcomings of your work. It is also important for you to explain how these limitations have impacted your research findings. Therefore, please revise the limitations of your study taking into consideration the following points:

- describe all limitations of the study and explain their relevance;
- explain the nature of the limitations and justify the choices you made during the research process;
- assess the impact of each limitation to the findings and conclusions of your study;
- suggest how the limitations could be overcome in the future.

Thank you for bringing up this important issue. Research aiming at elucidating embryological pathophysiology and immunology involving human tissue samples, moreover taken from children during reconstructive surgery is prone to a number of limitations. We acknowledge that the way we describe these limitations was not sufficiently comprehensive yet. According to your comments the respective passage at the end of the discussion section in the manuscript was adapted and partly rewritten (line 331ff). We tried to include all above-mentioned points, in order to render potential future perspectives for research clearer we also added two references. To us, it now seems much more coherent.

Furthermore the formal criteria and the editorial policies, including the availability of our original data (Zenodo, https://zenodo.org/record/1323160#.XL4Vey9XbUI) were reviewed.

The manuscript was proofread by a native English speaking professional in order to further improve on proficiency in the use of English language (comment made by Reviewer #1, Kentaro Mizuno).

Dear Reviewers!

We appreciate the fair and constructive comments you made and we would like to express our gratitude for your valuable work.

Please find a reply to each point raised below, all changes in the manuscript are in blue.

By addressing your comments, we feel that our paper has improved in clarity and quality.

We hope you share our view to have addressed all your points adequately.

Sincerely!

The authors
Kentaro Mizuno (Reviewer 1): General comments:
On the hypothesis that developmental differences in epithelial embryogenesis, the frequency or morphology and consequently also the function of epidermal and dermal Langerhans Cells (LCs) in patients with hypospadias, authors have investigated the number of LCs in the sheets and skin section derived from the patients with and without hypospadias. In the present study, LCs are present in similar frequencies and with comparable morphology and distribution in patients with hypospadias as compared to children without penile malformations.

Specific comments:
1. This paper is well-written, structured article. However, in the first place, is it likely that the number of LCs could be varied by the status of sample preparation, for example, past history of balanoposthitis?
This is a most important consideration. It has been shown in murine and human models, that various stimuli, including inflammation but also presence of TLR activators, can alter the status of epidermal and dermal LCs, also affecting their frequency by prompting migration. For our study, we assured to only include boys with clinically and histologically non-inflamed appearing foreskin and without a history of balanoposthitis. We did not mention this yet in our manuscript. A respective passage therefore was added to the methods section of the manuscript (line 106ff) and also to the results part (line 181).

2. Authors described that patients' characteristics are presented in Table 1. However I could not find out Table 1.
We apologize; Table 1 was not included into the initial submission by error. It has been added by now (line 420ff) and contains a detailed overview of the patient characteristics. Thank you for making us aware.

3. In discussion section, authors described that as there was no difference in LC frequency, we conclude that preoperative androgen application does not prompt LC migration by a topical inflammatory response. But, dermal or epidermal environment would be variable depends on the duration of preoperative hormonal therapy. Authors should indicated the lag time from hormonal therapy to hypospadias surgery in each case.
Thank you for your comment. In our clinical practice, 4 weeks lie in between the topical dihydrotestosterone application and surgery (and therewith sample acquisition).
We could not agree more that this is a critically important consideration. Therefore we added a respective passage, clarifying our approach to the methods section (line 115ff). In the discussion section we added a sentence with a more detailed reference to the cited literature (line 322ff and 328f) and a more accurate description of our conclusions.

Takanori Ochi (Reviewer 2): To examine dermal/epidermal Langerhans Cells in hypospadias patients was new and good trial. However, unfortunately, results of this paper is negative data as authors mentioned.
Thank you for your acknowledgement regarding our research hypothesis. We also felt, knowing that the timeframe of epidermal LC colonization and the development of hypospadias coincide and in view of the eminent role LC play in genital infections, that it would be worth to take a look. Furthermore, hypospadias are a very common diagnosis (1:125-300 males) and it became sufficiently clear during the last years (Gredler et al., Development, 2015) that also ectodermally derived tissues are involved.
As to our impression, finding no difference is a negative result with regard to the study hypothesis but
nevertheless important information deserving to be available in the literature body. Yet, there is no other publication on genital skin of children with penile malformations addressing this issue. Publishing this report would also enable us to make our data accessible online (Zenodo, https://zenodo.org/record/1323160#.XL4Vey9XbUI), helping to foster future research into the pathophysiology of skin in patients with genital malformations. To our understanding, further research is needed in order to eventually improve on the current surgical results for these boys.

Furthermore, to examine dermal/epidermal Langerhans Cells after birth might not be good enough to evaluate the relation to hypospadias. Of course, there is a limitation. We could not agree more. To answer the question of ontogeny and to examine how skin develops in children with genital malformations prenatally, our approach of looking at (postnatal) surgical specimens can only deliver indirect evidence. We further addressed these (and other) limitations in a rewritten passage at the end of the discussion part (line 331ff) and we acknowledge that this was not sufficiently clear beforehand.

To actually address this question in a human system (i.e. prenatal tissue sampling, embryological examination of hypospadias patients) is ethically and practically impossible. A way to do so would be an animal model of hypospadias (e.g. FGF10 or FGFR2 knockout mice, Reference 31) and an experimental approach similar to that of Tripp et al (Reference 18). We added this as a potential future perspective (line 326f).

Authors tried to consider the relation between the results of frequency/morphology of Langerhans Cells in hypospadias and HIV/HPV/Penile cancer in discussion, however, it was not the main part which the authors hypothesized in this paper and it was just a conjecture. Thank you for this comment. It is true that neither our results nor the data can be directly extrapolated to HIV/HPV and penile cancer. The immunology of skin in these boys, however, beneath the role it plays in wound healing, is primarily interesting in regard to HIV and HPV infection. The respective paragraphs in the discussion part were modified – the passage where we stated that “our results provide evidence that males affected by hypospadias carry the same risk of HIV infection compared to those who have no genital malformation” was edited accordingly (line 296 and 298f HIV and line 311 and 314f HPV). Furthermore we removed the respective passage from our conclusion (as well in the manuscript as in the abstract) as our results contain no data to directly support such a conclusion. We believe that the paper gained in clarity and objectivity by these edits and hope to have addressed your concern adequately.