Author’s response to reviews

Title: Adrenarche and Pubarche in Girls with Turner Syndrome during Growth-Promoting Therapy with Human Growth Hormone

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Author’s response to reviews:

To The Editor:

First we would like to thank the Editorial Board for the possibility to resubmit our paper. We thank you and the reviewers for their helpful comments. We have addressed all points of criticisms (see below) and highlighted the changes in the manuscript in yellow. We have reorganized some of the passages in the text.

Enclosed you can find the revised manuscript. We hope that you will now judge the paper suitable for publication.

Point-by-point response letter:

Editorial Comments:

1) Figure 1: We changed the group name “Normal girls” to “Control group”.

2) We indicate all statistically significant differences in Figure 1 and Table 1.
3) Statistics: We recalculated the data with the nonparametric Kruskal-Wallis test together with Dunn's Multiple Comparison Test. The Mann-Whitney U test was used to compare the ages at which the different Tanner stages of pubic hair were reached.

4) We include a section “Conclusion”.

5) We have revised the Declarations section.

6) We have no reference number for the ethics approval.

7) Written consent was obtained from all parents.

8) We changed the heading “Summary” to “Abstract” and removed the section heading “Objective” and integrated the text into the “Background” section and changed the heading “Patients and Methods” to “Methods”.

9) In the main text we changed the headings “Introduction” to “Background” and “Subjects and Methods” to “Methods”.

10) We added the full names of all authors on the title page.

11) We removed the figure titles and legends. The table legend was moved to below the table.

12) We used our own data on DHEAS levels as control group.

To Reviewer 1:

We revised the section “Methods”. We regret that some of the wording was unclear. Adrenarche is biochemically characterized by increasing serum concentrations of dehydroepiandrosterone (DHEA) and its sulfate conjugate DHEAS. We used our own data as reference data for the DHEAS levels. The DHEAS levels of the TS girls were measured with the same method. Your assessment that these are historic measurements is partially correct; however the data of the TS girls on GH treatment was also collected over a long period.

We moved the clinical data comparing PH staging with a reference population from the section “Discussion” to the section “Results”.

To Reviewer 2:

We have no reference number for the ethics approval. Written consent was obtained from all parents. We added the full names of all authors on the title page. We removed the figure titles and legends. The table legend was moved to below the table.

We used our own data on DHEAS levels as control group.

To Reviewer 3:

We revised the section “Methods”. Adrenarche is biochemically characterized by increasing serum concentrations of dehydroepiandrosterone (DHEA) and its sulfate conjugate DHEAS. We used our own data as reference data for the DHEAS levels. The DHEAS levels of the TS girls were measured with the same method. Your assessment that these are historic measurements is partially correct; however the data of the TS girls on GH treatment was also collected over a long period.

We moved the clinical data comparing PH staging with a reference population from the section “Discussion” to the section “Results”.

To Reviewer 4:

We revised the section “Methods”. Adrenarche is biochemically characterized by increasing serum concentrations of dehydroepiandrosterone (DHEA) and its sulfate conjugate DHEAS. We used our own data as reference data for the DHEAS levels. The DHEAS levels of the TS girls were measured with the same method. Your assessment that these are historic measurements is partially correct; however the data of the TS girls on GH treatment was also collected over a long period.

We moved the clinical data comparing PH staging with a reference population from the section “Discussion” to the section “Results”.

To Reviewer 5:

We revised the section “Methods”. Adrenarche is biochemically characterized by increasing serum concentrations of dehydroepiandrosterone (DHEA) and its sulfate conjugate DHEAS. We used our own data as reference data for the DHEAS levels. The DHEAS levels of the TS girls were measured with the same method. Your assessment that these are historic measurements is partially correct; however the data of the TS girls on GH treatment was also collected over a long period.

We moved the clinical data comparing PH staging with a reference population from the section “Discussion” to the section “Results”.

To Reviewer 6:

We revised the section “Methods”. Adrenarche is biochemically characterized by increasing serum concentrations of dehydroepiandrosterone (DHEA) and its sulfate conjugate DHEAS. We used our own data as reference data for the DHEAS levels. The DHEAS levels of the TS girls were measured with the same method. Your assessment that these are historic measurements is partially correct; however the data of the TS girls on GH treatment was also collected over a long period.

We moved the clinical data comparing PH staging with a reference population from the section “Discussion” to the section “Results”.

To Reviewer 7:

We revised the section “Methods”. Adrenarche is biochemically characterized by increasing serum concentrations of dehydroepiandrosterone (DHEA) and its sulfate conjugate DHEAS. We used our own data as reference data for the DHEAS levels. The DHEAS levels of the TS girls were measured with the same method. Your assessment that these are historic measurements is partially correct; however the data of the TS girls on GH treatment was also collected over a long period.

We moved the clinical data comparing PH staging with a reference population from the section “Discussion” to the section “Results”.

To Reviewer 8:

We revised the section “Methods”. Adrenarche is biochemically characterized by increasing serum concentrations of dehydroepiandrosterone (DHEA) and its sulfate conjugate DHEAS. We used our own data as reference data for the DHEAS levels. The DHEAS levels of the TS girls were measured with the same method. Your assessment that these are historic measurements is partially correct; however the data of the TS girls on GH treatment was also collected over a long period.

We moved the clinical data comparing PH staging with a reference population from the section “Discussion” to the section “Results”. We added the full names of all authors on the title page. We removed the figure titles and legends. The table legend was moved to below the table.
To Reviewer 2:

Methods and Results:

1. The type of karyotype did not influence the time of adrenarche/pubarche. We performed this analysis and have added this information in the section “Discussion”.

2. The mechanisms responsible for the initiation and maintenance of the adrenal secretion of androgens are poorly understood. There are many hypotheses in the literature. We could not assess the role of insulin resistance in earlier adrenarche/pubarche because we have no data. Serum IGF1 levels were elevated during GH therapy but always kept in the normal range (< 2 SDS). On GH therapy, the patients' weight was regularly analyzed and normal between the 10th and 90th percentile. The BMI had no influence on adrenarche.

3. We could not find a late adrenarche in TS girls. Adrenarche was exaggerated in all TS girls in comparison with the control group. Pubarche was delayed only in TS girls with ovarian failure.

Introduction/Discussion:

1. We cited your study published recently by Hankus et al. (Horm Res Paediatr 2018) in the paper (Discussion).

2. We clarified the discrepancies which you addressed and apologize for confusing wording.

3. We have no good idea to explain the higher DHEAS concentrations in TS patients. We addressed some hypotheses in the discussion. We speculate that higher IGF1 levels during GH therapy in TS girls might affect the adrenal cortex to produce more DHEAS. We have no data on Turner syndrome patients who have not undergone GH treatment.

To Reviewer 3:

We do not share your opinion that this paper does not add any novel information on this topic. Data on adrenarche in Turner girls in the literature is very scarce. Our data only partially confirm previous data published by the authors from Tübingen. If you enter the search strategy “adrenarche” and “Turner Syndrome” in PUBMed, you will receive 8 results. If you enter the key words “Growth hormone” and “Turner Syndrome” then you receive 1203 results.
Major points

1+2+3) We revised the presentation of the data (see section “Methods”). We clearly addressed that this was a longitudinal retrospective study. The whole cohort consisted of 94 girls and young women with TS who were treated with human growth hormone (GH). There was only one patient who was treated with GH from the age of 21 years (Tanner B1, bone age 12 years) until the age of 23.2 years. The longitudinal data of all patients were retrospectively ascertained from patient charts. The data collection ended in January 2016. The patients were divided retrospectively into two groups with regard to pubertal development: group 1 (n = 21) with spontaneous onset of puberty and group 2 (n = 70) with induced puberty due to primary ovarian insufficiency (POI). We excluded 3 patients from the analysis because they were too young to assess puberty at the time of the study.

4) The definition of adrenarche and pubarche was given in the section “Background”.

5) We add data on FSH levels in both groups (group 1: at Tanner B2; group 2: before starting therapy with estrogens) (section “Results”).

6) There was only one TS patient in the cohort who was older than 17 years. Therefore, we show only the DHEAS data of the age group 15 – 17 years in the results section and in figure 1.

7) Thank you for this important comment on statistics. We recalculated the data with the nonparametric Kruskal-Wallis test together with Dunn's Multiple Comparison Test. We used the Mann-Whitney U test to compare the ages at which the different Tanner stages of pubic hair were reached (see section “Statistics”)

8) We added the significant differences between the groups in Fig. 1.

9) We changed the form of the data presentation in Table 1.

10) We changed the statements in the conclusions (“Abstract” and section “Discussion”). We add data on the age at onset of menarche in both groups of TS girls.

Minor points

1) We changed the bibliography.

2) The manuscript was linguistically edited by an English native speaker.