Author's response to reviews

Title: Associations between speech features and phenotypic variability in Treacher Collins syndrome

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Author's response to reviews: see over
Dear Executive Editor,

Please find enclosed the revised manuscript to be resubmitted to BMC Medical Genetics.

Thank you very much for the review of the paper. The revised manuscript has been reformatted according to authors’ guidelines and manuscript template to conform the journal style. The requested information concerning the name of the regional ethics committee and the approval numbers have been included in the manuscript.

**Responses to reviewers’ concerns:**
The reviewers’ valuable comments have been addressed one by one and our responses are written in italics below.

Reviewer: Angela Morgan

**Minor essential revisions**
The research questions are well formed, yet it is important to alter the wording in the first question. The word variable implies one would be measuring speech at more than one time point. A better term could be the word similar...i.e., How similar are the speech features in TCS...across ages? This is just a small change that will remove any ambiguity and is arguably a truer representation of what was examined.

*See also the first line of the discussion where the word variability is used again.*

*The suggested alteration of wording in the first question has been made:*

**Background, page 6:** “How similar are the speech features ...?”

**Discussion, page 19, first to second line:** The word variability was chosen to be taken out and the new phrasing is: “The primary objectives of the study were to explore the speech characteristics associated with Treacher Collins syndrome in different age groups,...”

The methods are appropriate for addressing the research questions. The use of intra- and inter-rater reliability is appropriate and should be commended. Clearly a lot of rigorous work has gone into phenotyping this cohort of 19 participants. There is appropriate use of supplementary materials by inclusion of the rating scales used in the study to enable replication of the work.

Sample size and managing the sample: I had initial concerns about the division of the sample into paediatric and adult sub-groups when the sample was already small. Yet on reading the work, I can see that this was a clinically relevant approach. Indeed the findings indicate improvement in speech across the lifespan for individuals with TCS, including in the real-world outcome of speech intelligibility. Hence, I agree this was an appropriate approach for the authors to take.
There should be mention of the limitation of the sample size here in the discussion however, as of course we cannot be sure that another sample of just 19 participants would lead to the same conclusions until this work has been replicated. We could be more confident about the findings with a sample of 50 or greater. I did not see any mention of this. Thank you for including this in your revision.

**Discussion, page 26** - A new heading “Advantages and limitations of the study” has been included and the sentence “Interpretation of the findings must be careful due to limited sample size…” addresses this concern. Please notice that the first paragraph under the advantages and limitations heading is new text.

My only other query regarding sample size was the number of statistical tests performed. Should there have been a correction for multiple comparisons?

The authors have consulted a statistician and have followed the recommendations. Corrections for multiple comparisons have been carried out by changing the level of statistical significance:

**Methods, Statistical analyses, page 15** - The last sentence has been altered to “To reduce the risk of type 1 errors, \( p \leq 0.01 \) was interpreted as statistically significant.”

The change resulted in a few corrections of results and consequently several changes comments concerning statistical evidence. The following changes have been included:

**Abstract, Results, page 2-3** - “Anterior oral articulation errors were significantly associated with malocclusion…” has been deleted and the passage now reads: “The speech composite scores correlated with TCS severity scores and NOT-S total scores. Speech composite scores higher than 4 were associated with cleft palate.”

**Methods, Characteristics of the study group, page 9, second paragraph** - “Females had significantly higher severity scores than males (\( U=15.50, p=0.04 \)).” has been deleted.

**Results, Intelligibility, page 18** – The first sentence now reads: “The median percentages of intelligible and unintelligible words were significantly higher in the adult group than among children and adolescents (Table 3)”. Please notice the comment on change from mean to median percentage addressed in the response to the other reviewer below.

**Results, Speech composite score, page 18** - “Three of 11 adults and 6/8 children and adolescents were identified with four or more speech deviations. Seven of the adults presented with no or one single deviating speech characteristic. No significant difference between age groups was established (\( U=16.00, p=0.02 \)).”

**Results, Associations between structural malformations, hearing loss and speech, page 18-19** - The sentence “Anterior oral articulation errors, but not posterior oral substitutions, were significantly associated with malocclusion (\( p=0.02 \)).” has been deleted. The passage now reads: “…A significant association was found between speech composite scores higher than 4 and cleft palate (\( p=0.01 \)).” Significant correlation was found between hearing loss (PTA) and VPC-sum (\( \rho=0.57, p=0.01 \)).”

**Discussion, page 18, first paragraph** - The comment on statistical difference between the age groups has been taken out. The sentence now reads: “…Most of the adults presented with maximum one affected speech feature, while all but one of the children and adolescents displayed multiple deviations.” (Please also see response to suggested rephrasing below).

**Discussion, page 20, third paragraph, second and third line** - “…and a significant association was found between malocclusion and anterior oral substitutions.” has been deleted. The sentence now reads: “In the current study malocclusion, mainly increased maxillary overjet, occurred in nine participants.”

Something that was not clear to me? the methods read as though the individuals had the hearing tests on the 2 days of testing within this prospective speech study? Is that the case? I don’t think that would have been the case? But this is how it reads? so it sounds like hearing aids were fitted at that
timepoint. But I imagine that many of these individuals (particularly the adults) may have had hearing aids for up to decades?

The text has been clarified by adding the following information:

**Methods, Characteristics of the study group, page 8, third paragraph**

“Sixteen of 19 participants were using hearing devices. Six had bilateral bone-anchored hearing aids (BAHA) and two had unilateral. Seven had behind-the-ear (BTE) devices, five of which were unilateral and two bilateral. One participant was using bilateral in-the-ear (ITE) aids. Pure-tone audiometry (ISO 8253-1 1989) was used to update hearing measures.”

**Discussion, page 21, third paragraph, second to fourth line**

… “The current study group mainly comprised teenagers and adults, who to our understanding, had been using hearing aids since childhood.”

Introduction: It is difficult to get a feeling for the scale of past studies in relation to your sample of 19 participants. Please insert the sample N for the studies you mention. It is helpful to know the percentage of speech deficits in each group across these studies but difficult to interpret more broadly in relation to the field without the N.

Number of sample size has been inserted in the following sentences in the background:

**Page 4, second paragraph, lines 9-10**

“Compromised respiration at birth has been reported in nearly half of the 47 patients treated by the Australian Craniofacial Unit [10].”

**Page 5, third paragraph, first line**

“Another study has reported speech abnormalities in 74 percent of the 46 patients with TCS.”

- Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

Minor typographical: Characteristics of the study group, paragraph 3, last line? ?All devices were appropriately fitted, but two individuals with an unilateral aid were assessed as requiring bilateral devices. ? should be ?a unilateral aid?.

The text has been clarified:

**Methods, Characteristics of the study group, page 8, last in the third paragraph**

“All except two individuals had appropriately fitted hearing devices. Both were using unilateral hearing aids, but were assessed as requiring bilateral devices.”

Characteristics of the study group, under the Nordic orofacial screening test section - need to alter the following sentence to denote that there were issues with chewing and swallowing. As the sentence currently reads, it sounds like these basic skills were present rather than that individuals showed deficits in these skills. Chewing and swallowing was equally as frequent, but present in 6/11 adults, 3/4 adolescents and all four children.

The word “difficulties” was of course missing and has been added.

**Methods, Characteristics of the study group, page 9, third paragraph, line 11**

“Chewing and swallowing difficulties were equally as frequent (present in 6/11 adults, 3/4 adolescents and all four children).”

Discussion ? line 4 ? ?Most of the adults had no or only one affected speech feature,? ? suggest rephrasing this sentence. A little awkward for the reader to read ?no or only one?

The sentence has been rephrased to ease reading.
Discussion, page 19, first paragraph, lines 5-6- “Most of the adults presented with maximum one affected speech feature, while all but one of the children and adolescents displayed multiple deviations.”…

Discretionary:
Speech composite score: Some statisticians or bioinformaticians would query the use of a composite score. It is challenging to say that 1 point means the same thing across all of the measures you have included. I understand the rationale for this approach? to make comparisons more feasible. I feel this is probably acceptable given the authors have been so clear and specific about their intentions and methods. I felt I had to mention that I was a little unsure about this approach.

Do you have any basic information on the participants who declined to take part in your study? It would be helpful to determine whether you have a highly selected clinical sample, e.g., did only those individuals with more severe or less severe speech issues take part?

The issue of how representative the study group is for the Norwegian TCS population has also occupied the authors. We lack information particularly about adults that didn’t accept the offer of extended health examinations. This makes it impossible to predict how their participation would have influenced on the results. Due to this uncertainty would commenting on this in the report be highly speculative.

We like, however, to take the opportunity to share how we have reasoned: Our impression is that the eligible individuals that did not respond to offered extensive health examination were of two main categories. One comprised some children that had medical follow-up by different specialists in multi-disciplinary settings. The parents expressed their children did not require any extra healthcare services. The second category consisted of adults with no follow-up related to the craniofacial disorder other than services regarding hearing loss. A couple of adults contacted the corresponding author as coordinator of the project. They expressed that being approached as an individual with a syndrome had caused distress. They had a hearing problem and had no wish to be examined further. There may an additional number of adults with TCS unknown to the national healthcare services for craniofacial conditions.

The first and second authors are members of the National Craniofacial team. The impression is that children with diagnosed or suspected TCS are referred to the team from early in infancy. The number of unknown children with the condition should be very low and thereby minimizing the risk of a selected sample. However, the number of eligible adults is probably higher than the ones that we access via our patient registers, but it is impossible to anticipate neither the number nor the severity of their condition. Based on these experiences, one can assume that studying a larger sample may have lead to more evident differences between age groups and phenotypic severity.

Ethics: Do you have a specific approval number/numbers for your ethical approval? Would be helpful to cite that specifically in the paper. I will leave this up to the discretion of the Editorial team to consider and follow-up.

The requested approval numbers have been added:

Methods, Recruitment, page 7, first paragraph- The recruitment procedures and study protocol were approved by the Regional committee for medical research ethics at the University of Oslo (REK Sør-Øst A, approval number S-08553a) as well as the Norwegian Data Inspectorate at Oslo University Hospital (approval reference 05-2008).

Level of interest: An article whose findings are important to those with closely related research interests.
Quality of written English: Needs some language corrections before being published

Corrections of grammatical errors have been carried out by consulting a native speaker.

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests: I declare that I have no competing interests

Reviewer 2:
Anneli AY Yliherva

Reviewer's report:
Major Compulsory Revisions
none

Minor Essential Revisions
None

Discretionary Revisions
1. The introduction is well done but a little bit hard to follow for a reader with such a huge amount of information. One suggestion would be to make a table including all potential phenotypic expressions of TCS including structures in different areas of head and neck.

The authors consider that adding a table including all phenotypic expressions related to TCS would contain much the same variables that in Table 1. We have therefore chosen not to supplement with another table.

2. In background -section abbreviations are not used logically. For example Treacher Collins syndrome is sometimes written and sometimes abbreviated. This is the same even later in the manuscript. This is a small thing but careful reporting is important in scientific presentations.

The use of all the abbreviations listed in the Abbreviations paragraph on page 26 has been corrected.

3. The questions 1 and 2 are appropriate and well described, but the question number 3 is a little bit unclear because the speech is not defined. I have understood that the speech here means speech composite score including six different characteristics.

Background, page 6, third question- Speech has been defined by specifying speech features and the question now reads: “How are structural malformations, like malocclusion, cleft palate and hearing loss, associated with articulation errors, nasal resonance, velopharyngeal dysfunction, altered voice and intelligibility in TCS?”

4. There is missing information concerning the areas from where the TCS cases came. Were they living nearby Oslo area or were they also from other areas? The prevalence of TCS is low (1 in 50 000 live births), so in Norway there should be in the whole country approximately 100 TCS cases? In that sense the number of 19 cases in the study sample is sufficient even though the age groups of children?s and adolescents? are small including only 4 cases each.

Methods, Participants, page 7, first sentence- The participants were from different parts of the country and information on this matter has been supplemented: “The study group comprised 19 individuals from 14 families, 13 females and 6 males, geographically spread over ten of the nineteen Norwegian counties.”
5. When introducing the participants it would be interesting to know why the age groups were divided from 5 to 10 years, from 11 to 18 years and from 19 onwards. A professional knows that it is something to do with the growth but still justification for this would be useful.

**Methods, Participants, page 7, third sentence** - The division of the age groups was made due to speech developmental reasons. The third sentence of the paragraph has been rephrased: “The participants were divided into three subgroups due to potential influence of speech developmental factors: …”

6. One point which occupies is the use of parametric tests for testing normality (the Kolmogorov-Smirnov test (K?S test) which is a nonparametric test for the equality of continuous, one-dimensional probability distributions that can be used to compare a sample with a reference probability distribution (one-sample K?S test), or to compare two samples (two-sample K?S test)). But I suppose the authors could explain and justify this question.

The authors appreciate that reviewer pointed out the statistical error. The statistics regarding intelligibility have been recalculated according to instructions given by a statistical advisor:

**Methods, Intelligibility, page 14, last sentence in the first paragraph**- “The median value of the three ratings was reported as the final result for each participant.”

**Methods, Statistical analyses, page 15, lines 5-7-** “…The Mann-Whitney U test was applied to test independence between age groups. Correlation was tested using Spearman’s rank correlation.”

Consequently the following changes were made in the manuscript:

**Abstract, page 3, first paragraph**- “The percent of intelligible words in connected speech was significantly lower in children and adolescents (median 77%, range 31-99) than in adults (98%, range 93-100). Intelligibility of speech among the children was markedly inconsistent and clearly affecting the understandability.”

**Results, Intelligibility, page 18, first and second line**- “The median percentages of intelligible and unintelligible words were significantly higher in the adult group than among children and adolescents (Table 3).”

**Results, Intelligibility, page 18, lines 4-6**- “Three of the four youngest participants presented with the lowest median percentages of intelligible words with 31%, 59% and 67% respectively.”

**Tables, Table legend, page 32**- “Table 3 Percent of intelligible, unintelligible and omitted words in connected speech in individuals with Treacher Collins syndrome”

**Table 3, page 34**- Please notice the altered table text and insertion of recalculated results.

6. I couldn’t find the titles of the figures, and also the abbreviations should be opened in all figures.

**Figure legends are written on pages 31-32.**

The abbreviation (PCC) has been supplemented in the legend for Figure 1 (page 31). The abbreviation “TCS” has been added last in the Table 1 legend (page 33).

7. The discussion is quite extensive and also includes a lot of comments and suggestions on clinical applications. This is really a good thing but I would have put all the clinical suggestions in the end of the discussion or in the conclusion.

In addition, there was a little bit repetition of results in some points.

**The reviewer’s proposition to put all clinical suggestions at the end of the discussion was considered, but restructuring the discussion would have caused an unfortunate imbalance and was therefore rejected. The intention of bringing results into the discussion is in the authors’ opinion helpful for the reading.**

In conclusion the authors state that adults should be followed but what about children and adults?
Conclusions, page 26, lines 4-8-- The importance of monitoring speech in children with and providing intervention has been highlighted by rephrasing the conclusions: … "The complexity of speech problems identified in young individuals with TCS indicate that speech monitoring and intervention are required from early in life preferably in multidisciplinary craniofacial team settings. A subgroup of adults with persisting speech deviations may require prolonged attention of speech language pathologists."

And how we could know what kind of phenotype especially is important to follow more carefully.

Discussion, page 24, second paragraph- The issue regarding what kind of phenotype especially is important to follow more carefully has been intended to address by emphasizing that clinicians should recognize that the more severely affected individuals are the more important likely it is that they have problems in different functional areas: in the paragraph:

“The low percentage of intelligible words among the youngest participants underlines the need to facilitate speech and communication skills. Considering the high prevalence of deviating speech features and hearing loss, early referral for assessment of speech function and communication is recommended. Early introduction of sign language is often useful to augment communication and language development. One should recognize that the speech composite scores were correlated with the TCS severity scores and the NOT-S scores, which express the close inter-relationship between structural malformations and different orofacial functions [19]. It is important that clinicians realize that identification of speech aberrations in individuals of all ages with TCS is a clear indication to perform assessments of respiration and food intake and vice versa. “

What could be the signs of speech characteristics to screen in TCS cases etc. For a SLT all practical and concrete information is always very useful. What would be also useful is to summarize the information more concretely to help clinicians to follow TCS cases carefully.

One purpose of the paper was to point out that speech deviations in TCS are complex and variable of character. Aspects of articulation, nasal resonance and voice as well as an evaluation of intelligibility need to be included in speech assessment of individuals with TCS for substantiated speech therapy planning.

8. I think it would have needed more discussion about the limitations concerning the children and adult groups (N=4 each). The authors put them in the same group but children between 5 to 10 and 11 to 18 years are different but how? They were first studied separately but I think they should have been discussing if this is the developmental group? The lack of information concerning the criteria how the groups were divided is essential here to report.

Concerns about the limitations of study have been addressed in a new paragraph “Advantages and limitations of the study” (page 26). Please see the question number 5 above regarding the reason for division of age groups. Also notice that the first paragraph under the advantages and limitations heading is new text.

9. The title and abstract convey what has been found although the speech function/characteristics were also related not only to phenotypic variability/severity of TCS but also orofacial dysfunction measured by NOT-S. In that sense it should be: Association between speech features and phenotypic variability OR severity and orofacial function OR dysfunction in Treacher Collins syndrome. The title is not really wrong but a little bit imprecise (see also Background in Abstract).

The authors agree that there was a mixture of terms in the original manuscript. The issue concerning precision of title and objective was discussed with the clinical geneticist involved in other parts of the current project. The conclusion was that “phenotypic severity” covers the different aspects of observed
features related to a condition, i.e. dysmorphic features, hearing loss, orofacial dysfunction, and correctly describes the objective.

The following changes have been made in the revised manuscript:

**Title:** “Associations between speech features and phenotypic severity in Treacher Collins syndrome”

**Abstract, Background, page 2, last sentence:** “Exploring speech features and investigating if speech function is related to phenotypic severity are essential for optimizing follow-up and treatment.

**Background, page 6, first paragraph:** “The objectives of the present study were to explore speech features associated with TCS, and to investigate how these speech characteristics are related to phenotypic severity of the condition.”

**Discussion, page 19, lines 2-3:** “… and to investigate if speech function was correlated with phenotypic severity of the condition.”

**Conclusion, page 26, line 3-4:** “Speech dysfunction was significantly correlated with phenotypic severity of TCS.”

In abstract section and in the methods the authors express they have 19 cases between 5 to 74 years, but there is a wide gap between 19 and 29 years of age. Maybe it would have been useful to report 19 cases in three groups: 5-10, 11-19, 29-74 years of age? The last group really concerns the grown-up people. There were really adults > 29 years.

**Abstract, Methods, page 2, first sentence:** The sentence has been change according to the reviewer’s suggestion as following: “Articulation, nasal resonance, voice and intelligibility were examined in 19 individuals (5-74 years, median 34 years) divided into three groups comprising children 5-10 years (n=4), adolescents 11-18 years (n=4) and adults 29 years and older (n=11).”

**Methods, Participants, page 7, third sentence:** “…: children 5-10 years (n=4), adolescents 11-18 years (n=4) and adults from 29 years and older (n=11).”

**Figures 1, 2A, 2B:** “≥ 19 years” has been changed to “≥ 29 years”

**Table 1, Table 2 and Table 4:** “≥ 19 years “has been changed to “Adults”

Hopefully the revised manuscript will be satisfactory and found to be worthy of publication in BMC Medical Genetics.

Yours sincerely,

Pamela Åsten,
Speech-language pathologist, on behalf of the authors