Author's response to reviews

Title: Facial Morphometrics of Children with Non-Syndromic Orofacial Clefts in Tanzania

Authors:

- Mange Manyama (manyama73@yahoo.com)
- Jacinda R Larson (jrlarson@ucalgary.ca)
- Denise K Liberton (dklibert@ucalgary.ca)
- Campbell Rolian (cprolian@ucalgary.ca)
- Francis J Smith (fsmit@ucalgary.ca)
- Emmanuel Kimwaga (emmanuelkimwaga@hotmail.com)
- Japhet Gilyoma (drgilyoma2@yahoo.com)
- Kenneth D Lukowiak (lukowiak@ucalgary.ca)
- Richard A Spritz (Richard.Spritz@ucdenver.edu)
- Benedikt Hallgrimsson (bhallgri@ucalgary.ca)

Version: 4
Date: 23 May 2014

Author's response to reviews:

May 23, 2014

Dear Editors,

We are resubmitting our revised manuscript, “Facial Morphometrics of Children with Non-Syndromic Orofacial Clefts in Tanzania”, for consideration as an article in BMC Oral Health. We conducted a 3D geometric morphometric study comparing facial shape variation in a group of Tanzanian children with cleft lip with or without cleft palate and a group of normal controls. To our knowledge, this is the first such study carried out in an African population, and shows that facial shape differences associated with clefts in Africa is generally similar to that in Caucasian populations.

We previously submitted this manuscript (and assigned manuscript ID# 7621633531092677) and received feedback from the editor and several reviewers. The primary concern with the original manuscript was sample heterogeneity and limited sample size. We have increased the sample size of the case group from 33 to 44 individuals and the control group from 33 to 88. Additionally, we standardized each group to the same age to better compare variation across the groups.

Regarding the figures: We have replaced figure 1 which was based on actual person with another figure that is based on average shape (after morphing). In this new figure, landmarks that were not part of this study were covered with oval shapes. Figure 2, 4, 5 are based on average shape (after morphing) of actual individuals in the unrepaiored CL/P and Control groups. We obtained consent from all these individuals.
Finally, we now compare patients with isolated cleft lip and patients with cleft lip/palate, finding no significant difference in effect of cleft type on surface facial morphology. Below is a point-by-point response to reviewers:

Reviewer report 1 (nikolaos pandis):
Comment: -please use only 2 decimals points for p-values except in the cases where p<0.001
Response: We have used only 2 decimal points for p-values (except where p<0.001).
Comment: "between left-sided unilateral CL/P and bilateral clefts (Procrustes distance=0.0708, p=0.0224) (Figure 3)." page 7. You indicate this as nonsignificant
Response: This part has been re-written after the analysis was re-done
Comment: please include in the discussion a paragraph about the limitations of the study design (cross-sectional), potential biases and confounders and sample size
Response: We have mentioned in the last sentence on the discussion section that small sample size was a limitation in our study and its implication.

Reviewer report 2 (Piotr Fudalej)
Comment: The study has a methodological deficiency, which affects both findings and conclusions – the sample is very heterogeneous. The CL/P group consists of subjects with various types of cleft: cleft palate only (CP), unilateral cleft lip and palate (UCLP) and bilateral cleft lip and palate (BCLP). All were shown to have various degrees of growth disturbances. Furthermore, the age range of the sample is very wide – from 2 months to 10 years. Various surgical techniques used for cleft repair and various timings of operations (usually very late from a perspective of European or American cleft team) used in the treatment make the sample even more heterogeneous. Unfortunately a good technique of image acquisition and adequate method of data analysis cannot overcome the problem of the heterogeneity of the sample.
Response: The primary concern with the original manuscript was sample heterogeneity, particularly given the small sample size at the time of submission. We realize the importance of being able to accurately describe facial shape variation in our sample and have adjusted our sample and results accordingly. Foremost, we have updated the sample size of the case group from 33 to 44 individuals and the control group from 33 to 88. We now have a 2:1 ratio of cases to controls, and also enough samples within each group to more accurately test and control for the ontogenetic effects on facial shape within each group. Additionally, we standardized each group to the same age to better compare the variation across the groups. The affected group of our sample had individuals with isolated cleft lip and patients with cleft lip/palate, finding no significant difference in effect of cleft type on surface facial morphology. Finally, due to the
fact that various surgical techniques used for cleft repair at various timings of operations increases the heterogeneity of the sample size, we have therefore excluded individuals with repaired CL/P in our sample size.

Reviewer Report 3 (Demetrios Halazonetis)

Comment: The Abstract does not contain any numerical results. The authors should add statistical data of their most important results.
Response: We have added statistical data for the most important results in the abstract.

Comment: Sample: "33 were gender- and age-matched controls": I fail to see how this is true. The male/female ratio of the controls was 1.5 whereas that of the CL and CLP groups was 0.6 and 1.4, respectively, or 0.8 for the whole cleft group.
Response: We have deleted the sentence "gender- and age-matched controls" and we have increased the control group to 88.

Comment: The authors do not provide data on when the clefts were repaired, relative to the timing of records. How much time had elapsed between repair and 3D scanning? Such data are essential to ensure that the conclusion: "surgical repair of CL/P in this population failed to provide a completely harmonious facial appearance" is valid.
Response: We realized (after receiving a comment from one of the reviewers) that including the repaired CL/P was making our sample size more heterogeneous. We have therefore excluded this group from our sample size.

Comment: "Six standard views were obtained for each subject": These might not have been taken with the patient at the same posture (soft tissues or mandible), so merging of the views might have entailed error. How was merging achieved in such cases? How did the authors deal with (even slight) differences in soft tissue morphology between different views, due to posture, strain, etc.?
Response: We have included the error that could result from the merging process in the morphometric data section.

Comment: How did the authors deal with patients who had the lips apart? Where was point 5 located in such cases?
Response: In cases where lips were apart, we tried as much as possible to place landmarks to the nearest point where the expected landmark will otherwise be located.

Comment: "Standardization of the dataset for age and size was then carried out using multivariate regression": So, was there any allometry? Ontogenetic differences? What were the results of the regression?
Multivariate regression on age and size for this period of development (2 months to 10 years) may not be appropriate. Regression assumes that the variables are
linearly related, which might not be true here. The authors should provide some evidence of linearity. Was centroid size used for the regression or did the authors transform to log?

Response: Standardization age has been redone using a different statistical analysis and results of regression described (as shown under section "shape analysis").

Comment: Were unilateral clefts flipped to one standard side? From the results (Figure 3), it seems that this was not done. Please clarify. If no flipping was performed then it is surprising that the authors do not describe any PC that related to asymmetry of the un repaired CL/P group (first paragraph of the Results section).

Response: To compare unilateral versus bilateral clefts as a group, all individuals with right-side clefts were mirrored to have left-side clefts, by inverting the sign of the x-coordinate for all landmarks. Comparisons of left versus right, however, were carried out on the data prior to mirroring. This is highlighted in the manuscript under "shape analysis section"

Comment: The authors do not report who identified the landmarks on the scans. Was only one observer used?

Response: Only one observer was used to identify the landmarks. This has now been indicated "morphometric data section"

Comment: "or between left-sided unilateral CL/P and bilateral clefts (Procrustes distance=0.0708, p=0.0224)": Is this P value correct? If yes, then it is below 0.05, therefore statistically significant.

Response: This analysis has been redone and the anomaly has been corrected as indicated in the result section.

Comment: Figure 2B shows 22 females (red dots) but Table 1 reports 21. Also, 10 males are shown whereas Table 1 reports 12. A similar problem occurs in Figure 3, where, e.g. 6 bilateral patients are shown, but Table 2 reports 5.

Response: The whole analysis have been redone after exclusion of the repaired group and increase of the control group. Similarly, the corresponding figures have also been redone and the anomalies therefore corrected.

Comment: Figure 4C: The extremes of PC1 seem to be of different overall size (the (-) shape seems much larger than the (+) shape both anteroposteriorly and transversally). Is centroid size the same?

Response: The data were subjected to Procrustes superimposition to rescale to standard size, translate to standard position and rotate to standard orientation. Therefore the centroid size is the same.

Comment: "PC2 is associated with positioning of the maxilla, especially in a retrognathic manner, as well as cranial base and nasal width": It is strange to mention the cranial base when no skeletal structures could be visualized and when no points were placed on the cranial base. The authors should reword this,
and other occurrences of the term.
Response: By cranial base width we meant distance between one tragus to the other. However, we now agree with the reviewer and have deleted cranial base from our descriptions.

Comment: "The Mahalanobis distances between individuals who had isolated CL and combined CLP were significant..., whereas the Procrustes distances between these groups were not significant": The authors should explain what each of these two metrics signifies, and why they showed conflicting results.
Response: We have redone the analysis and therefore the anomaly has been rectified.

Comment: Table 1 should be augmented with the age of the patients (average, SD, range of each group).
Response: This information has been added in the text in the method section under "sample"

Comment: Table 2: "Midline inflection point where the frontal and nasal bones connect": these are soft tissue points so bones are not visible.
Comment: Table 2: "inflection point": Please look up the meaning of this term (e.g. http://en.wikipedia.org/wiki/Inflection_point). It is not used appropriately here.
Comment: Table 2: "The minima of the superior margin of the tragus": 'minima' is plural. Also, what exactly does this mean?
Response: We have made the corrections as shown on table 2

Comment: Figure 2C and 2D: I don't understand the bottom-most row. Are these the landmarks as seen from the lateral aspect? Which side is the front of the face? Are landmarks 23 and 24 represented in these line drawings?
Comment: Figure 2C and 2D: What is the range in the shape plots? Are these the extremes of the sample?
Response: All the figures have been re-done with all the line drawing replaced with morphs for more clarity.

Comment: Figure 2B: It would be nice to color the dots according to laterality and type of cleft.
Comment: "Separation of the three groups can be seen in the CVA results (Figure 5)": This should be corrected to 'Figure 6B' and '6D'. Actually, the whole paragraph is confusing and Figure 7 does not seem to be discussed at all.
Response: We have re-done all the figures have addressed the above raised comments.

Comment: "include a short and relatively large nose": This seems like a contradiction in terms, please elaborate.
Response: We have corrected this as shown in the highlighted sentence.

Comment: Discussion: "the CL/P group was characterized by increased interorbital distance": the authors should keep in mind that this distance was increased, but only relative to the other distances (due to Procrustes alignment); it does not follow that this distance was actually larger in the CL/P group. Therefore, the discussion concerning interorbital distance should include this caveat.

Response: We have re-written the discussion part and the interorbital distance is discussed in relation to other distances.

Comment: "Even following corrective surgery, individuals with orofacial clefts have been reported to display facial shape differences...": Most probably 'because of' corrective surgery.

Response: In order to reduce heterogeneity in our sample, we have excluded individuals with repaired CL/P.

Reviewer report 4: Edwin M Ongkosuwito

Comment: The authors should also elaborate on a study that has a similar kind of setup and has not been mentioned in the introduction Weinberg SM, Naidoo SD, Bardi KM, Brandon CA, Neiswanger K, Resick JM, Martin RA, Marazita ML. Face shape of unaffected parents with cleft affected offspring: combining three-dimensional surface imaging and geometric morphometrics. Orthod Craniofac Res. 2009 Nov;12(4):271-81

Response: We have elaborated and included by Weinberg et al. in the discussion section.

Comment: Are the methods appropriate and well described? (major compulsory revision:). The method is appropriate for these kind of data. However with a lot of methods, all results depend on what is initially entered into the model.

Is the variation in the shapes of the different clefts (bilateral versus unilateral) not too big to put them in one group? The steps are quite well described. However no basic data (means, sd’s ) other than the overall age data are described. I cannot find when the sample was collected. The boundaries set in the tests are not reported, also the assumptions made to implement the data are not completely described.

Response: We have included the time when data was collected as well as other basic data (range, SD). We have also described the assumptions to implement the data.

Comment: In the results p values are mentioned but it doesn’t mention before what the statistical boundaries are. Such as the transformations to one standard size or the standardization to one age. How do we know that these transformations do fit the data well? These are not reported.

Response: Standardization to one's age has now been done as shown in the highlighted section
Comment: (minor essential revision) No software is reported for the procrustes, PCA and CVA 3.
Response: We have now mentioned the software used for data analysis.
Comment: PCA re expresses the data linearly. This makes it easier to make an interpretation out of many data entries. (Major compulsory revision: )For facial shape variation within the unrepaired group PC1 explains 22.7% of the model PC2 explains 15 %. However PC 1 is so broad and PC2 seems to be a part of PC1 that they don’t seem to relate to logical anatomical structures. Why do we have these outcomes here and can they not be more specific like in the other groups?
Response: The analysis has been re-done to address this comment.
Comment: Is the writing acceptable? Yes I feel that a statistician should review the methods and advise what should be reported in every step and which assumptions that were made should be reported.
Response: The analysis has been redone accordingly.

We thank the editors and reviewers for their suggestions. We believe that we have now addressed their concerns, and that these changes have greatly improved our study, resulting in a stronger manuscript. We hope that you agree and now consider our revised and rewritten manuscript acceptable for inclusion in BMC Oral Health.

Thank you,
Mange Manyama