Author’s response to reviews

Title: Growth from birth to two years in a cohort of children diagnosed with CF by newborn screening

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Author’s response to reviews:

Dear Editor

Many thanks for the reviewer’s helpful and supportive comments. We have addressed each of the points in turn.

Reviewer 1

• The cohort spans from 2007 - 2014. Growth measurements are compared against WHO standards. It would be useful to have a matched control group from the same population, but given this is unlikely to be available, the authors might discuss the possibility that a control group from this geographic location might also demonstrate z-scores on anthropomorphics that are less than zero. In other words, is the z-score for mean birthweight of a non-CF cohort from the same region not negative.

The reviewer is correct in his assumption that we do not have a matched control group from the same population. We now mention this in the limitations section of the discussion.

• The methods section provides very little detail on how the "clustering algorithm" and was carried out (making the interpretation of the supplementary table difficult.

The methodology for the clustering algorithm is given in the referenced paper. We purposefully didn’t include it in the methodology as we did not think it relevant to the target audience (clinicians) and feared it may put readers off continuing to read the article. We are happy to be advised by the editorial board if they want us to include more detail.

• The methods section also says little about the classification model analysis was constructed and carried out. This type of analysis has not been commonly used, so the reader needs more guidance. There are no statistics presented, and there is no ranking of the importance off the variables, relative to each other, in predicting ultimate outcomes. Were other models attempted? What is the strength of the data of the models presented relative to each other? There are no classification model data presented for analysis by genotype (508 homozygotes vs. other). Does this mean the variable was trialed but not
significant, or did the authors not evaluate genotype by the classification method. As with the point above, we were trying to balance giving enough detail on the classification model whilst still keeping the whole article relevant to the target audience. This is the only modelling we used. The model analyses all the inputs but only put those with a predictive effect into the decision trees. We have clarified this in the methodology.

• Means are presented for growth parameters, but it would be interesting to know whether data on median values yielded similar results. Means were presented as the data was normally distributed so the medians were similar.

• What were the mean/median ages at which fecal elastase was measured. pseudomonas/staph was cultured? Fecal elastase was always measured at the first clinic appointment. This is now clarified in the methods section. We have added a separate Microbiology Data section into the results which gives details of the Pseudomonas and Staphylococcus isolates.

• The discussion should address why the differences in growth were less in the second year of life. This is now mentioned in the discussion.

• Use of antibiotics and other specific CF medications is not discussed or factored into modeling - rather just that standard protocols for treatment were followed. Are these data available and might they be integrated? Is treatment so consistent that these variations are unlikely to be important contributors? We do not have comprehensive data on treatment throughout the first two years of life so this could not be included in the model. Treatment is consistent but we have now listed this as a limitation in the discussion.

• Did gender impact the modeling outcomes? It did not. We have clarified in the methodology that all input were assessed by the model but only those that were predictive of growth parameters were used in the decision trees.

• When discussing presence of pseudomonas, are there data about mucoid/non-mucoid status, at what age the cultures first turned positive, whether treatment was instituted and whether the culture remained positive over multiple samples?

All first isolates were non-mucoid. All children who isolated Pseudomonas underwent eradication and none of them met the Leeds criteria chronic PA infection during the first two years of life. This is included in the results section as is the relevant reference.

• Were there many births in the cohort <37 weeks? If so, other growth curves (eg Olsen, Fenton) may be more appropriate for determining z-score, and this could impact the final average z-score. How many preterm infants were present in the cohort? 8 children were born at a gestation <37 weeks. For consistency it was felt that the same growth curves (WHO-UK) should be used for all children.

• There was no discussion of the possible role of IGF from the pancreas that might impact in utero growth.

Thank you for pointing out this important omission. We have now included this in the discussion with a new reference.
• Is it possible to discuss the data further in a framework of those CF infants in the cohort who responded to aggressive nutritional support vs. those who did not grow well? Might risk factors be different in the slow growing group?

Although this is an interesting suggestion the retrospective methodology means we do not have detailed enough data on the nutritional support to look into this accurately. This is now mentioned as a limitation in the discussion.

• Figure 1. needs a better legend that explains A, B, C, D - what they are and why they are different
The legend has been changed to ‘Decision tree models generated by classification modelling showing the factors predictive of mean weight and height z scores in the first and second year of life.’

Reviewer 2

1. The title "Growth from birth to two years in a cohort of children diagnosed with CF by newborn screening" is too broad, and did not reflect the main objectives of the study. In fact, growth patterns from birth to two years were not the main focus of the study.
We have changed the title to ‘Factors affecting the growth of infants diagnosed with CF by newborn screening’

2. Page 5 line 87 - "the remaining 129 children": the number seems not right (144 - 5 - 2 - 6 =131).
The article was correct. 15 children were excluded (144-5-2-2-6)

3. Page 5 line 95 - feeding type: please clarify that "breast" refers to exclusive breastfeeding.
We have clarified this as suggested

4. Page 6 Line 115-116 & line 115-116: The four outcomes were the mean z-scores for height and weight in the first and second year of life. Please provide justifications for using the mean values of the entire year, instead of the actual measurements taken at age 1 and 2 years, respectively. As we all know, growth during infancy is dynamic and non-linear. For example, a healthy infant's birth weight doubles by age 6 months, and triples by age 1 year. The average values will not capture such dynamic changes. Also, please explain why growth indexes weight-for-length and BMI are not examined.
The outputs were chosen as a way of summarising a years’ worth of z scores. The z scores varied significantly over time meaning a z score from a single time point was not representative. This has been clarified in the methodology. We wanted to keep the methodology as simple as possible so chose to just use height and weight as outputs.

5. Page 6 line 104 - 105: Please explain why these cutoff points "-2, -1, and 0" were chosen. Moreover, not every child would "achieve" these values. What would be the time to achieve these cutoff values for these children?
Using whole number z scores seemed a logical choice. We accept not every child achieved all these cut-off but wanted to give some representation to the readers regarding the timeline of catch-up growth.
6. Page 7 Line 125-130: Please report some of the characteristics of the study population, i.e., phenotype distribution (MI, PI, nonMI-PI), percent of infants born prematurely, percent of infants with low birth weight, genotype distribution, age at the first clinic visit. Also, the number/percentage of heterozygous for deltaF508 seems wrong (122/129).
We have added the additional data on characteristics as suggested. In the results section we had used age at diagnosis instead of age at first clinic visit. This has been changed to avoid confusion. The number of children heterozygous for Phe508del is correct.

7. Pancreatic status changes over time. Please specify when pancreatic status was assessed.
Pancreatic status was assessed at the first clinic visit. This has been clarified in the methodology.

8. Page 7 Line 133-135: This statement is a bit misleading, as the test is not statistically significant. In fact, boys weigh more at birth than girls by ~100 gram in healthy, non-CF population (WHO growth reference).
We agree this was misleading and have therefore changed the results section so that the birth weights and birth weight z scores are now given for boys and girls with no statistical comparison.

9. Page 8 Line 136: Please specify exclusive breastfeeding if it is the case.
Changed as suggested.

10. Appendix: it would be interesting to compare these two clusters with respect to distributions of phenotype, genotype, gender, % of premature infants, % of infants with low birth weight, age at the first clinic visit, age at the CF diagnosis, etc.
The clusters include genotype. We did not include the number of children born prematurely as we had included gestational age. The age at first clinic visit and gender were included in the cluster analysis but were very similar in the two clusters.

11. Page 8 Line 155-157: Some of the variables change over time, i.e., fecal elastase, infant feeding, respiratory infections. Please specify when these variables were assessed, and explain how they were defined. The classification models could be biased without considering the changes of these variables during infancy.
The retrospective methodology meant that it was not possible to collect data on all possible factors particularly those that may have changed over the two years. We have discussed this in the limitations section of the discussion.

12. Page 8 line 161: please define "poor infant growth".
We have changed this sentence to: ‘Classification modelling is also used to identify the variables affecting infant growth’
Editorial Comments

• We have added a Declarations section as requested.
We hope that by addressing all the reviewer’s comments the article can now be accepted for publication.

Kind regards

Yours truly

Dr Francis Gilchrist

Dear Editor

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