Author’s response to reviews

Title: Protocol for assessing maternal, environmental and epigenetic risk factors for dental caries in a population of Queensland children

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Author’s response to reviews:


Dear Editor,

RESPONSE TO REVIEWERS

We thank the reviewers for reading our manuscript so carefully and for their thoughtful comments.

Dr Haas has written “The paper describes the methodology of an ongoing study of determinants of dental caries in 6 years old children from Australia. The methods are well described and suitable for the type of study” and has recommended acceptance without revision.

Dr Polk has written: “The writing is generally clear and the topic is relevant for the BMC Oral Health audience. The level of detail provided in most of the sections was appropriate and sufficient.” She has asked for attention to three major aspects, and has raised 16 minor points for our consideration.
The Editor’s decision is for minor revision, before sending to reviewers.

We are extremely grateful to Dr Polk for the care and attention she has given to our manuscript. We have addressed all these matters below and believe, as a result, that this is a considerably improved paper and, indeed, improved project.

Major Points:

1. Please provide a rationale for why we need more risk factors for oral health. At lines 85 through 93, you list many risk factors for oral health. If the purpose of the study is to identify additional risk factors, you should provide an explanation for why the existing risk factors are not sufficient.

Prediction of risk for disease enables, at a population level, focussed and potentially more cost-effective health promotion: at an individual level, more cost-effective screening and early intervention. Many factors influence susceptibility to dental caries, and their relative strength varies between populations, so the extent to which our study finds common, and new risk factors is important.

Most importantly, the influence of epigenetics on susceptibility to dental caries is currently totally unknown. Given the clear, strong, influence of host genomic polymorphisms on susceptibility to caries, it is likely that the epigenome also has strong influence. This is highly original work.

2. Please provide a conceptual model. There are many, many variables being assessed in this study. I need to see how you think they all fit together. You may decide to narrow the focus of the study to address things that can cause methylation and relationships between methylated genes and disease.

Thank you for the suggestion. We have constructed a conceptual framework, which is now included as Fig 1.

3. Please provide more detail in data analysis section. The data analysis section needs a lot more detail. See examples of published protocols (listed below) for examples of data analysis sections. I think having a conceptual model will help with this section. Once you have a conceptual model, then you can think about mediators and moderators/confounders and make sure your analysis treats them appropriately. I'm also really concerned about the number of analyses that could potentially be conducted. Will p-values be adjusted to account for this? Or will some other strategy be adopted?

Thank you for providing these helpful examples from the literature. Our plans for statistical analyses have been considerably expanded. See new paragraphs in the text.
Minor points

1. In the Abstract, lines 38-39. The sentence describing the birth cohort beginning in 2012 and six year old children is unclear because children born in 2012 are not yet six. The language used at lines 145 and 154 later in the manuscript is clearer, stating that the children were six in 2012.

This was an error: Corrected thank you.

2. Lines 49 to 52, Discussion. The Discussion section of the Abstract is written as though this manuscript is a proposal and not a protocol.

This is indeed intended as a protocol document. We have tried to make the origins of the study in an existing birth cohort study clear, indicate what preliminary cross sectional data have been used to design the protocol and its detailed methodologies, and lay out future work. We have sought to use appropriate tenses, in the grammatical sense, for each of these aspects.

3. Somewhere in the Abstract, perhaps in the Methods section after the first sentence, it should be stated clearly that this is a cross-sectional study.

This has been made explicit.

4. Line 90. I'm not sure "Conversely" is the right word. Perhaps "Additionally?"

Agreed. Changed.

5. Line 106, inherited alterations in sugar metabolism. Although I agree that there probably are inherited differences in sugar metabolism, I think the direct, topical effect of sugar on the oral flora is far more important for the caries process.

It is undoubtedly true that dietary sugars, especially but not exclusively sucrose, play a major role in the aetiopathogenesis of dental caries. The topic is receiving a great deal of attention in both lay and professional media at present, following recent publications from the World Health Organisation. However this does not exclude a role for the way an individual handles sugars, including genetic influences - or even predetermination. Hereditary fructose intolerance is a clear example.

Such an explanation has been added at lines 100 - 122 and the Tang et al reference (Ref 43 specifically), added to make this point. Thank you for the suggestions

7. Line 153, Sample recruitment. This section also describes the examination study participants undergo. You might add that to the section title.

Done.

What happens if you see disease during the examination? Do you give referrals for treatment? Please address this.

We certainly offer care to all needy mothers and children [the dental treatment is free for the latter] and this has been made explicit in the revised manuscript.

8. Lines 154 through 157. Do you have any information about differences between families that accepted and families that declined to participate? Also, has the representativeness of the study sample relative to the population been retained?

Pragmatically, this has to be opportunistic recruitment from the original birth cohort. We have sought to make it easy for families from all geographical locations to attend our clinic by offering appointments outside of school and sometimes working hours, and providing free car parking. Post hoc, it will be possible to address the question of the degree to which the sample is typical of south east Queensland as a whole, for example in terms of spread of family income and family size.

There is always a risk of bias with volunteer studies, in that parents/families who acquiesce may be amongst the more health conscious.

9. Line 166, Figures 1a and 1b. I think you can combine boxes and reduce the flow chart to one figure. See examples of published protocols (listed below) for examples of flow charts.

This has been done.

10. Lines 173 to 182, Sample size. This gets back to my major concern about the lack of information about the statistical analyses. You say the sample size is based on multivariate regression model with up to five variables, but in the data analysis section, you don't commit to this analysis. Also, would this sample size be true even if you conducted 100 regression models with up to five variables? Or is it true only if you conduct one regression model with up to five variables? Given all the analyses you presumably will be conducting, this sample size seems surprisingly small to me.

This is explained in detail in an expanded section on our likely statistical approach. We can collapse predictor variables into fewer categories when, a priori, they are conceptually interrelated. Further, as is always the case, statistical approaches can be modified depending on initial results.
Whilst we would like larger numbers, this is not possible in terms of time and cost [lines 200-201]. We are, however, comforted by the fact that our pilot epigenetic screen on the 12 dyads described in lines 264-267 show statistically significant differences between high caries and low caries families [data not shown in this protocol document].

11. Lines 183 to 192, Primary outcome variable. As written, you don't actually commit to a primary outcome variable. Also, how is the severity of restored lesions determined? It doesn't seem like it can be determined. So then untreated caries severity is primarily a measure of how long the carious lesions have been around. Do you have any hypotheses about reversible versus nonreversible lesions?

The primary outcome variable is dental caries experience of children.

We are using ICDAS II criteria to assess the caries status. ICDAS gives caries scores from 0 (no caries) to 6 (extensive pulp exposed cavities). The index is sensitive in the sense that it detects initial white spot lesions (1 and 2) and minute enamel lesions (3). The severity can be evaluated in many ways;

I. No caries

II. Low caries experience (scores 1-3)

III. High caries experience (scores 4-6)

IV. Total current caries experience (1-6)

V. These can be expressed in numbers, percentages or as a ratio against healthy tooth surfaces

In addition to the detection of carious lesions, ICDAS assesses the presence of treated caries, in the form of the presence and type of restoration on any/every tooth surface. We will take restorations as;

I. Past caries experience to include in total caries experience which, will be expressed as a percentage of total tooth surfaces

II. Past caries experience, will be explored as a risk indicator for total caries experience, especially current untreated lesions

The matter of “severity of restored lesions” is fraught, and has never been solved in epidemiological studies. In all existing caries indices, including the universal DMFT/S and dmft/s, a restored surface is scored as carious on the assumption that a clinician has determined that it was carious, and required to be restored. However this depends on the clinician’s individual judgement [eg is a minimal intervention approach used, permitting/encouraging remineralisation of non-cavitated lesions, or has the clinician adopted a traditional “drill and fill” approach?]; has “extension for prevention” been practised?; has the restorative
technique/material chosen necessitated extension for mechanical reasons? These are unavoidable
caveats to this type of research. Nevertheless it is theoretically possible to search for the quantum
of error which such ambiguities might inject into the results by taking different ICDAS score
cut-offs in the statistical analyses, as mentioned above.

The reversibility of non-cavitated lesions is now well established. This can be achieved by
clinical interventions such as topical fluorides and artificial remineralising solutions, gels or
mousses. The best remineralising solution is the patient’s own natural saliva because it is
supersaturated with calcium and phosphate ions at the pH of secretion, is at the right
temperature, and is free! The mineralisation – demineralisation equilibrium can only work in
favour of the tooth, however, if the local pH is not depressed by acid generated from the
metabolism of simple sugars by bacteria, and the dental biofilm is thin enough to allow ions to
permeate. We measure the cariogenicity of the oral environment by our tests of salivary
physiology and explore their relationship to cumulative caries, always taking cognisance of the
fact these are taken at a single point in time, not over the life course of the individual. Examining
their predictive value for future caries activity is outwith a cross-sectional study design.

12. Lines 194 to 196, Main explanatory variables and Lines 197 to 200, Oral health knowledge
and practice. Please provide more information about these measures. See examples of
published protocols (listed below) for examples of the level of detail for measures. With
respect to oral health practices, specifically what is being measured? Oral hygiene, sugar
consumption? Other factors?

We have provided this extra detail in lines 217-226 of the revised manuscript.

13. Lines 202 to 207, Anthropometric measurements and Lines 218 to 224, Periodontal status.
Please provide a rationale for why these measures are being taken. Do you have hypotheses
about weight and timing of tooth eruption, for example?

We take BMI and waist circumference of mothers as a measure of their dietary behaviours and
life style. There is a literature to show, in some populations, that obese mothers have children
with higher caries rates. Obese mothers tend to have more obese children.

Indirectly these measures are also a reflection of the dietary pattern of the child, which in turn
affects risk of dental caries.

Diet and lifestyle have a profound effect on the epigenome.

Periodontal status of mothers (PSR) is again an indirect measure of oral hygiene, of oral health
behaviours, and of any genetic susceptibility to oral disease. (The child’s periodontal tissues are
also examined as part of the comprehensive head, neck and mouth examination: any
abnormalities are noted and the child referred for management. Because periodontal parameters
tend to be normal in children of this age, detailed recordings of gingival status, calculus and pocket depth are not made).

14. Lines 226 to 238, Demographic and environmental data. How will you handle it in the data analyses if families change over time, such as if mother's educational status changes for example?

It is not possible to take a comprehensive life course approach in this study, for every aspect of the mother’s and the child’s environment and behaviour. It would be dangerous to enlarge the number of explanatory variables beyond the power provided by the size of the sample. Nevertheless, longitudinal data are available from the main IFHL database of changes in family environment since birth, and of child health throughout his/her life, including childhood diseases, antibiotic use and hospital separations. These are included in the conceptual model which we have now provided. The power to explore the effects of these as predictor variables will only become apparent as data accumulate.

15. Lines 240 to 257, Genetic and epigenetic markers. As I understand it, this manuscript is describing the study protocol. If the protocol hasn't yet been determined for the genetic and epigenetic markers, then it may be premature to publish

We have revised this section to explain that the protocols for both the DNA extraction and purification and DNA sequencing have been established: The former in our laboratories with these methods currently being written up for publication elsewhere. The latter have been published in Allum et al, 2015.

We have also now indicated that our initial pilot study has been used to establish the necessary bioinformatics methods required for this project.

And it also states that results from the pilot study will be used to define a sample size for a large scale future study. Yet in the Sample size section earlier in the manuscript, the case was made for a sample size of 147. This is confusing.

We agree that referring to sample size again here was confusing and have addressed this by removing the statement from this section and expanding the section entitled: "Sample Size".

We have described further the potential value of modern epigenomics to understanding disease susceptibility in Background and provided much more detail of our approach to epigenetics in lines 264-288. The sample size of 147 dyads is a pragmatic approach to this study of multiple variables in the study overall.

We believe this clarifies this issue.
The pilot study of 12 dyads from whom the epigenome has been sequenced has, as stated in the response to item 10, already shown statically significant differences. These findings will inform the number of pairs which could be sequenced to reach significance for other genomic sites.

16. Table 1. How are the data in this table organized? It might make sense to organize them by caries experience. And if you have data about the caries experience of study participants, you could add that as another column.

We think it premature to add such data from but part of the cohort in a protocol paper, which is not intended as a results paper.

Once again we thank Dr Polk for her careful critique, from which we have gained much benefit.

Sincerely, Newell Johnson and colleagues.

Gold Cost, Australia, 25 September 2015