PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Cohort profile: Aichi regional sub-cohort of the Japan Environment and Children's Study (JECS-A)
AUTHORS	Ebara, Takeshi; Yamada, Yasuyuki; Shoji, Naoto; Ito, Yuki; Nakagawa, Atsuko; Miyachi, Taishi; Ozaki, Yasuhiko; Omori, Toyonori; Suzuki, Sadao; Kojima, Masayo; Ueyama, Jun; Tomizawa, Motohiro; Kato, Sayaka; Oguri, Tomoko; Matsuki, Taro; Sato, Hirotaka; Oya, Naoko; Sugiura-Ogasawara, M; Saitoh, Shinji; Kamijima, Michihiro

VERSION 1 – REVIEW

REVIEWER	Aimin Chen University of Cincinnati USA
REVIEW RETURNED	27-Dec-2018

GENERAL COMMENTS	This is a summary of cohort profile: JECS-A in Aichi region, part of the larger JECS cohort but with some distinct features. The description of the cohort was well drafted and the major demographic characteristics were provided in detail. A few comments are given to improve the summary profile. 1. The enrollment strategy can be more detailed, particularly in the sense of missing low SES women in the area. The reason of this missing and the potential bias in life style factors can be provided. It is unclear whether incentives were provided for the enrollment, and whether the informed consent and questionnaire survey occurred in separate days. Was transportation an issue? The maternity care facility can be described in more details, for example, did the enrollment occur in selected prenatal care facilities? How about gestational age at enrollment and follow-ups during pregnancy (if at all)? 2. The common elements from JECS and the special features from JECS-A can be delineated better. For example, using different marks in Table 1. Regarding environmental exposures, what are the plans within the JECS or JECS-A for chemical assays? Biospecimen volume of each sample type can be provided. Storage and retrieval of biospecimens can be described better, and the data management issues can also be briefly discussed. 3. Two interesting attempts are diaper urine extraction and finger length comparison. It is probably premature to argue that 2D:4D ratio is more predictive of anything while the current screening tools may need a physician's participation. For urine extraction, what are the frequency of collection? For 2D:4D ratio, does that involve both hands?

	4. Even though 45% of JECS-A cohort were housewives, did pregnant employees also come to prenatal clinics during day time? Or night or weekend clinics were available for them? 5. Birth length is more commonly used rather than height. 6. Table 1 may need a grid to facilitate the reading.

REVIEWER	Diana Kuh Professor of Life Course Epidemiology UCL UK
REVIEW RETURNED	24-Feb-2019

GENERAL COMMENTS	Re Ebara et al. Aichi regional adjunct sub-cohort of the Japan Environment and Children's Study (JECS-A) This paper describes the complex study design of a sub-cohort (JECS-A) of the Japan Environment and Children's Study (JECS) which has 5 adjunct studies as well as the main study linked to the JECS. While it has many interesting features, such as new chemical exposure assessments, the text was hard to follow and needs many clarifications (as indicated in the points below). The study was established in 2011 but the only findings reported are the basic demographics. Thus the interest lies in the scientific questions it plans to address and/or the gaps (methodological or otherwise) in the existing literature it plans to fill. These are vague and not very well presented. This paper will require major revisions. Abstract 1. The abstract mentions parents but only mothers appear to be recruited. Are the fathers involved in the study and the data collection? 2. The abstract appears to suggest that the data mentioned are collected on all cohort participants but the main text suggests that there are 5 different adjunct studies, each collecting different types of data. Introduction 3. First paragraph, p.5. The interest in the effects of the fetal, perinatal and childhood environment on the health of children at birth and during later life has not recently become 'an issue', as suggested by the first paragraph on page 5. Research showing the importance of these environments for child health has been recognised for many years and research showing their importance for adult health has been widely published since the 1980s. Please rephrase. I think you may mean environmental chemical pollutants, as mentioned later. 4. Clarify how this regional sub-cohort relates to and interacts with the main study of other regional cohorts in terms of samples, scientific questions etc. This is needed in the first paragraph on p,5 and when discussing the establishment of JECS-A (starting line 40, p.7). 5. Given that the JECS-A was established in 2011, why are the research themes 7+ years later only described as "possible"? 6. In the first sentence of para 2, page 5, who is paying special attention to neurodevelopmental disorders – the Aichi study, the JECS generally or are you simply referring to reference 2 (APA diagnostic manual)? 7. On first line of page 6 please give location of the cohort study of 7-year old children.

	8. Line 28, page 6, suggest delete 'On the other hand' as there is no mention of 'on the one hand' and the phrase is unnecessary. 9. So what are the gaps that this sub-cohort can address in the research on the effects of environmental pollutants? The one mentioned is the methodology for exposure assessments. Are there other gaps to be filled? 10. Are the screening tools being discussed in in introduction for ADHD, disabilities more generally or what? 11. The second and fourth objectives of the JECS-A require further clarification. How could maternal interactions modify the effects of chemical exposures on neurodevelopment (i.e. by what mechanisms? Are you testing the validity of the various screening tools (in which case what for) or only their "availabilities"? Cohort description Setting – should this be setting and recruitment to sub-cohorts or something similar? 12. A map of the region would be helpful for international readers 13. It is not clear whether the participants in sub-cohorts A1, A2 and A3 are mutually exclusive, designed as a hierarchy or simply 3 different adjunct studies that mothers could opt into or not. How much participant overlap is there between them? In other words, how do the numbers in each of these sub-cohorts add up to 5555, taking into account withdrawals, loss to follow up, abortion and stillbirths? 14. Make clearer that the second recruitment at 18 months was for a new set of adjunct studies? Recruitment for Sub-cohort B apparently followed after recruitment for sub-cohort C which makes for a little more confusion. 15. A clearer description of the consent process overall is needed. The authors suggest that for sub-cohort B written informed consent was collected from each child which is unlikely given they were 18 months old at the time. Patient and public involvement 16. Are the annual open lectures for participants well attended? Have other forms of feedback been considered? Data Collection and measurements 17. This section also needs renaming as it discusses some of the questions to be addressed in the different adjunct studies. 18. Please clarify whether it is only the socio-demographic data snd height, weight, BMI and sex that are collected for the main study? All the other data seems to relate to one or other of the adjunct studies. 19. " The adjunct studies were designed for four specific main objectives" (lines 11-12, p.11) How do these objectives link to the four study objectives noted at the end of the introduction? What are the hypotheses being tested? The discussion on the uses of these adjunct studies is rather vague on the scientific questions and in some cases lacks details of the methods to be employed, and needs to be tightened up. The neurodevelopment outcomes are part of the "main data" of the JECS – some explanation and proper referencing if needed to make sense of the adjunct studies. Findings to date 20. This may be more appropriately labelled 'Participant Characteristics' as there are no other findings presented. 21. Please make clear the implications of having low representation of low income households in the cohort. For example, how does this affect the type of scientific questions that

	can be addressed or how any findings can be generalised to the general population? 22. If possible, please provide a test of the differences between the cohort characteristics and the national characteristics rather than relying on visual inspection.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Aimin Chen
Institution and Country: University of Cincinnati, USA
Please state any competing interests or state 'None declared': None declared

Sincerest thanks for your comments on our manuscript. We have revised the paper in response to the extensive and insightful your comments. We have also added additional explanations to address your comments as follows.

1. The enrollment strategy can be more detailed, particularly in the sense of missing low SES women in the area. The reason of this missing and the potential bias in life style factors can be provided. It is unclear whether incentives were provided for the enrollment, and whether the informed consent and questionnaire survey occurred in separate days. Was transportation an issue? The maternity care facility can be described in more details, for example, did the enrollment occur in selected prenatal care facilities? How about gestational age at enrollment and follow-ups during pregnancy (if at all)?

The Setting section was rewritten, and the requested information was added under the new session heading 'Enrollment strategy' according to your suggestions. We also added potential information on factors being biased and possibly affecting the generalizability of findings. Interpretation of possible potential bias was provided in the Limitation section.

2. The common elements from JECS and the special features from JECS-A can be delineated better. For example, using different marks in Table 1. Regarding environmental exposures, what are the plans within the JECS or JECS-A for chemical assays? Biospecimen volume of each sample type can be provided. Storage and retrieval of biospecimens can be described better, and the data management issues can also be briefly discussed.

We clarified the common elements from JECS and the special features from JECS-A using different marks in Table 1. Regarding environmental exposures, we specified the plans within the JECS or JECS-A for chemical assays in the Introduction section. We also added the information on biospecimen volume of each sample type, storage and retrieval of biospecimens in text and in Table 1.

3. Two interesting attempts are diaper urine extraction and finger length comparison. It is probably premature to argue that 2D:4D ratio is more predictive of anything while the current screening tools may need a physician's participation. For urine extraction, what are the frequency of collection? For 2D:4D ratio, does that involve both hands?

We thank Professor Aimin Chen for the comments. As you pointed out, it is premature to argue that 2D:4D ratio is more predictive and available as a screening measure. In the revised manuscript we rewrote the Introduction section concisely and precisely. We measured the 2D:4D ratios of the children on both hands. We added the explanation in the Data collection and measurements section.

4. Even though 45% of JECS-A cohort were housewives, did pregnant employees also come to prenatal clinics during day time? Or night or weekend clinics were available for them?

Thank you very much for your valuable comments. It is one of the important points to discuss the representativeness of the study population. In Japan, many pregnant employees can generally take paid leave during daytime to come to prenatal clinics but not all the women can. Of course, some clinics are available at night and on weekends, unfortunately, we had no choice but to call on pregnant women for participation who had visited the obstetric facilities mainly during the daytime, owing to limited number of staffs during the evening shift at hospitals. We revised the relevant description in the Limitation section.

5. Birth length is more commonly used rather than height.

We replaced 'height' with 'length' in Table 3.

6. Table 1 may need a grid to facilitate the reading.

I agree with your suggestion. We will request the editorial office to make a grid for Table 1 in proof.

Reviewer: 2
Reviewer Name: Diana Kuh
Institution and Country: Professor of Life Course Epidemiology, UCL, UK
Please state any competing interests or state ‘None declared’: None declared

We greatly appreciate the time and effort you have dedicated to providing insightful feedback on ways to strengthen our manuscript. We have incorporated changes that reflect the constructive suggestion you have graciously provided. We have also added additional explanations to address your comments as follows.

Abstract
1. The abstract mentions parents but only mothers appear to be recruited. Are the fathers involved in the study and the data collection?

Thank you for your comments. The JECS-A covers pregnant women (n=5,721) and their children (n=5,555) in principle, and part of their spouses provided data as well since the main study of the JECS targeted both of parents (fathers' participation was optional, but suggested). Regarding the sub-cohort of JECS-A, we partially collected fathers' blood (optional). We added the information in the text in COHORT DESCRIPTION, and in Table 1.

2. The abstract appears to suggest that the data mentioned are collected on all cohort participants but the main text suggests that there are 5 different adjunct studies, each collecting different types of data.

This article mainly focuses on the cohort profile and its features of the Aichi regional sub-cohorts of the JECS-A, containing 5,721 pregnant women and their 5,555 children. In this context, we added 'as adjunct study of JECS', to clarify the relationship between the sub-cohorts and the adjunct studies. We also rewrote and restructured it in Introduction and Cohort description section.

Introduction
3. First paragraph, p.5. The interest in the effects of the fetal, perinatal and childhood environment on the health of children at birth and during later life has not recently become ‘an issue’, as suggested by

the first paragraph on page 5. Research showing the importance of these environments for child health has been recognised for many years and research showing their importance for adult health has been widely published since the 1980s. Please rephrase. I think you may mean environmental chemical pollutants, as mentioned later.

As you pointed out, it has not recently become an issue. We rephrased the sentence correctly.

4. Clarify how this regional sub-cohort relates to and interacts with the main study of other regional cohorts in terms of samples, scientific questions etc. This is needed in the first paragraph on p,5 and when discussing the establishment of JECS-A (starting line 40, p.7).

We partially reconstructed and corrected the Introduction section following your suggestion, especially the description in the first and last paragraphs of Introduction to clarify the features and relationship between the sub-cohort and the main cohort. We also clarified the common elements from the main study of the JECS and the special features from JECS-A using different marks in Table 1.

5. Given that the JECS-A was established in 2011, why are the research themes 7+ years later only described as “possible”?

Thank you very much for your comment. As you pointed out, the expression ‘possible’ was inappropriate. We deleted it.

6. In the first sentence of para 2, page 5, who is paying special attention to neurodevelopmental disorders – the Aichi study, the JECS generally or are you simply referring to reference 2 (APA diagnostic manual)?

We corrected the sentence to make it clear, following your comment.

7. On first line of page 6 please give location of the cohort study of 7-year old children.

As requested, we added the location as follows; ‘cohort study of seven-year-old predominantly Mexican American children in California's Salinas Valley…’

8. Line 28, page 6, suggest delete ‘On the other hand’ as there is no mention of ‘on the one hand’ and the phrase is unnecessary.

We deleted ‘On the other hand’, following your exact suggestion.

9. So what are the gaps that this sub-cohort can address in the research on the effects of environmental pollutants? The one mentioned is the methodology for exposure assessments. Are there other gaps to be filled?

We revised the Introduction section to clarify the mutually complementary relationships between the sub-cohort and the main cohort.

10. Are the screening tools being discussed in in introduction for ADHD, disabilities more generally or what?

As you pointed out, the objective of the study outcome to be discussed was ambiguous. We corrected the relevant sentences.

11. The second and fourth objectives of the JECS-A require further clarification. How could maternal interactions modify the effects of chemical exposures on neurodevelopment (i.e. by what mechanisms? Are you testing the validity of the various screening tools (in which case what for) or only their “availabilities”?

We totally reconstructed and corrected the Introduction section following your suggestions. We also rewrote the text in 'Data collection and measurements for adjunct studies of the JECS-A' section in response to the three objectives stated in the Introduction.

Cohort description
Setting – should this be setting and recruitment to sub-cohorts or something similar?

The Authors guidelines of this journal require to use designated structure of headings. "Cohort description" needs to describe the setting. We divided the paragraph into 'Setting' and 'Enrollment strategy' and rearranged relationship between nature of the main cohort and the sub-cohorts. We also clarified the consent process for the sub-cohorts.

12. A map of the region would be helpful for international readers
As requested, we added the map of study areas covered by the JECS-A as Figure 1.

13. It is not clear whether the participants in sub-cohorts A1, A2 and A3 are mutually exclusive, designed as a hierarchy or simply 3 different adjunct studies that mothers could opt into or not. How much participant overlap is there between them? In other words, how do the numbers in each of these sub-cohorts add up to 5555, taking into account withdrawals, loss to follow up, abortion and stillbirths?

We thank Professor Diana Kuh for the valuable comments. As you pointed out, it was obscure for readers. Sub-cohorts A1, A2 and A3 are designed as a hierarchy, that is, the participants in sub-cohort A3 meet the requirements of sub-cohorts A1 and A2. We added the explanation in COHORT DESCRIPRION section and revised Figure 2 to make it clear.
About the number of children, of the 5,721 pregnant women for main study of the JECS covered by the Aichi regional center, 3,424 participants (59,9%) agreed to participate in the JECS-A adjunct studies (questionnaire survey only), defined as the sub-cohort A1.
5,555 children mean the number of live births after excluding Withdrawal and Lost to follow-up (n = 113) and Abortion and stillbirth (n = 102), but including 49 pairs of twins. Finally, the number of children adds up to 5,555. We revised Figure 2 to clarify it.

14. Make clearer that the second recruitment at 18 months was for a new set of adjunct studies? Recruitment for Sub-cohort B apparently followed after recruitment for sub-cohort C which makes for a little more confusion.

To make them clearer, the description order of sub-cohort B and sub-cohort C in text and Figure 2 was changed.

15. A clearer description of the consent process overall is needed. The authors suggest that for sub-cohort B written informed consent was collected from each child which is unlikely given they were 18 months old at the time.

Thanks for your important comments. As you pointed out, we added the description of the consent process for the sub-cohort B and C, as follows; ‘the informed parental consent from the legally authorized representative was obtained for all the enrollment of children in the studies.’

Patient and public involvement
16. Are the annual open lectures for participants well attended? Have other forms of feedback been considered?

There are about 100 participants every year to the annual open lectures. We also mail a newsletter to introduce participants to research activities and its progress three times a year.
We have carefully considered the Reviewer's advice, but the section was quite lengthy, so that unfortunately, we omitted the description in the revised manuscript. However, if you think it would be better to have the description in the PPI section, we are willing to add it in a subsequent revision.

Data Collection and measurements
17. This section also needs renaming as it discusses some of the questions to be addressed in the different adjunct studies.

This section has been renamed 'Data collection and measurements for adjunct studies of the JECS-A'.

18. Please clarify whether it is only the socio-demographic data and height, weight, BMI and sex that are collected for the main study? All the other data seems to relate to one or other of the adjunct studies.

We clarified the common elements from JECS and the special features from JECS-A using different marks in Table 1.

19. " The adjunct studies were designed for four specific main objectives" (lines 11-12, p.11)
How do these objectives link to the four study objectives noted at the end of the introduction? What are the hypotheses being tested? The discussion on the uses of these adjunct studies is rather vague on the scientific questions and in some cases lacks details of the methods to be employed, and needs to be tightened up. The neurodevelopment outcomes are part of the "main data" of the JECS – some explanation and proper referencing if needed to make sense of the adjunct studies.

We totally restructured the 'Data collection and measurements' section following your suggestion, especially in response to the three objectives stated in the Introduction.

Findings to date
20. This may be more appropriately labelled 'Participant Characteristics' as there are no other findings presented.

The Authors guidelines of this journal require to use designated structure of headings. We set the subheadings 'Participant characteristics of pregnant women' and 'Participant characteristics of children' in FINDINGS TO DATE section.

21. Please make clear the implications of having low representation of low income households in the cohort. For example, how does this affect the type of scientific questions that can be addressed or how any findings can be generalised to the general population?

We sincerely appreciate your valuable comments. The reviewer 1 also pointed out the similar comments, so that we added potential information on factors being biased affecting the generalizability of findings. Interpretation of possible potential bias was also provided in the Limitation section.

22. If possible, please provide a test of the differences between the cohort characteristics and

the national characteristics rather than relying on visual inspection.

When the sample size increases this leads to that even very small effect sizes can become statistically significant. We provided the 95% confidence intervals in Table 2 and Table 3, for comparisons with the national statistics, since results about statistical significance are also possible with the help of the confidence intervals.
(that is, if the confidence interval does not include the value of the national statistics, it can be assumed that there is a statistically significant result).

Thank you again for your valuable comments and suggestions.

VERSION 2 – REVIEW

REVIEWER	Aimin Chen University of Cincinnati, USA
REVIEW RETURNED	30-May-2019

GENERAL COMMENTS	The authors have adequately addressed prior review concerns. Thanks!