**ARTICLE DETAILS**

**TITLE (PROVISIONAL)**
Birth cohort study on the effects of desert dust exposure on children's health: Protocol of an adjunct study of the Japan Environment & Children's Study

**AUTHORS**
Kanatani, Kumiko; Adachi, Yuichi; Sugimoto, Nobuo; Noma, Hisashi; Onishi, Kazunari; Hamazaki, Kei; Takahashi, Yoshimitsu; Ito, Isao; Egawa, Miho; Sato, Keiko; Go, Tohshin; Kurozawa, Youichi; Inadera, Hidekuni; Konishi, Ikuo; Nakayama, Takeo; Japan Environment & Children's Study, Group

**GENERAL COMMENTS**

The effects of desert dust particles on health are of interest, particularly for pregnant women and young children. The publication of a study protocol can be interesting as well, if it is well presented and useful for other researchers. I have major concerns about this particular protocol, its design and/or the way it is presented.

1. The purpose of the paper is not stated, in a concise and clear way, at the end of the introduction. A clear statement of the purpose, such as “informing the relevant research community”, would immediately bring some of the weaknesses of the paper into light.
2. The three health outcomes to be studied are not adequately described in the objectives. For example: the reference time for the development of allergies or asthma for infants is not specified in this section, but later stated as being ages 4-6. It should be included here. Similarly for the “outgrowing asthma for asthma infants” (I understand this as meaning asthma exacerbation for asthmatic children). The first outcomes (development of disease) may be the result of exposure over the whole pregnancy period and the whole life of a child (a longer time effect), the latter (exacerbation of asthma) is probably the result of short term exposure. It seems that the outcomes to be studied for pregnant women are also possible responses to short term exposures, but these characteristics are not clear in the description.
3. The second objective (“perform further analysis…”) is also not clear. One would say that it is about the effect modification potential of avoidance behaviors and vulnerability characteristics. This is not clear and it is further confused by the statement about “…what in the dust exerts its effects”, which prepares us for analyses of the
chemical composition of dust, an issue not addressed in the rest of the paper.

4. Methods and analysis. The section entitled “setting”, tells extremely little about the setting, i.e. the three locations that will be studied. Are they urban? What is the population? Is there air pollution apart from desert dust? Most important: is there particulate matter pollution? Is the “dust season” the same for all the areas? What is the usual PM concentration and how much is it during dust days?

5. Sample selection. The time frame is given but the characteristics of the enrolled pregnant women are not. At which stage of pregnancy are they? Will they have the possibility of being exposed during the subsequent dust season?

6. Study design. If the publication is going to be interesting for the research community, the full questionnaires should be included as an appendix. Table 1 aiming (I think) to provide information on the variables to be studied, is not informative, because it does not include the measurement of each variable. Additionally, how many control days per exposure day will be selected?

7. Sample size. For the primary purpose (asthma development- here referred as primary objective for the first time) the power calculations assume that there is a definition of low exposure levels, which does not appear in the paper. The second alleged power calculation (for worsening of allergic symptoms) is not presented at all in numbers.

8. Measurements. Demographics. More information is needed in this section and, particularly, which variables will be considered as confounders.

9. Exposure. Here lies the main weakness of this paper. The description of the exposure is very important, as this is the novel characteristic of the adjunct study and relates to the main hypotheses. The description is completely inadequate. Choosing days with high concentrations of desert dust and defining these as exposure days and choosing control days (presumably with low levels of desert dust- this is not mentioned but may be inferred- , but within the dust season- are there really such days?) allows comparisons relevant to the short term health outcomes, such as worsening of symptoms in pregnant women or asthma exacerbation in an asthmatic child. Nothing is mentioned on the calculation of long-term exposure, which is necessary to assess the “primary outcome” (please see point 7 above). Clearly there is no definition of how this long-term exposure will be classified as low or otherwise.

10. Exposure. Is this method validated? Are there any measurements of particles in the study areas?

11. Outcome. Please see point 6 above.

12. Confounders should be carefully described and they are not!

13. The data analysis methods are not mentioned at all, although they should be an integral part of a protocol.

14. In the discussion, the “automatic random selection of questions” is not explained.

15. The main discussion item, namely what are the potential confounders, including PM from other sources, is not touched. This needs to be addressed.

Minor comments.

The reference on which the authors are basing their statement that 40% of PM is from desert dust, is old and based on a model. Better evidence can probably be provided. The paper will need English editing.

This version of the paper does not describe the intended statistical analyses. If there is a revised version than a statistical review may
This manuscript reported a protocol of ongoing birth cohort study in three regions of Japan to explore the effects of Asian dust exposure on children’s asthma and allergic diseases. The study is adjunct with a large cohort study, The Japan Environment & Children’s study (JECS), with a total of 100,000 children across 15 regions in Japan. The authors established online questionnaire delivery system to participant's mobile phones and/or personal computers on dust and non-dust days linked with the Light Detecting and Ranging (LIDAR) system which was used to define the dust days. The study enrolled 3,302 pregnant women by the end of 2012 and continues to enroll subjects up to March 2014.

Overall, the paper is well written and the design sounds relevant. The web-based questionnaire linked to the atmospheric monitoring system allowing for quick data collection can be a useful tool for researchers in this field. I have some minor issues to be raised.

1. Methods and analysis: It would be informative for readers if the authors could provide a data analysis plan in the paper. As summarized in Table 1, the study plans to collect many variables of individual characteristics, symptoms, behaviors etc. in a repeated manner for some of the variables, as shown in Figures 2 and 3, on dust and non-dust days. The readers may want to know how these variables can be incorporated in the analysis to answer three research questions on page 8. Specifically, the readers may be confused how the variables of the day-to-day symptoms and that for the allergic disease development can be combined or separate in the analysis.

Minor comments

2. Title: As is guided in the journal instructions, I suggest to include the specific study type (e.g., cohort study) in the title.

3. Setting: Although the authors provided references for the JECS design, some brief explanation of the eligibility criteria, if any, to participate the JECS would be helpful especially for readers who are unfamiliar with the Study.

4. Measurements: line 52, page 11. Any reason to define the cutoff of the LIDAR desert dust measurement at 0.07/km? In addition, which altitude of the LIDAR measurement will be used? If it is based on the findings of the author’s previous study, probably the citation of the reference 17 here might be appropriate.

5. In the Table 1, “25(OH)D serum/immunoglobulin serum/inflammatory cytokines” is on the list and they seem to be measured by blood samples, but there is no description about it in the main text.
The effects of desert dust particles on health are of interest, particularly for pregnant women and young children. The publication of a study protocol can be interesting as well, if it is well presented and useful for other researchers.

I have major concerns about this particular protocol, its design and/or the way it is presented.

Thank you for reviewing our manuscript. Your comments were meaningful and helped improve the quality of our manuscript.

1. The purpose of the paper is not stated, in a concise and clear way, at the end of the introduction. A clear statement of the purpose, such as “informing the relevant research community”, would immediately bring some of the weaknesses of the paper into light.

Thank you for your comment. We revised the Introduction section to clearly set forth the purpose of the study, as suggested (page 8, line 110-113).

2. The three health outcomes to be studied are not adequately described in the objectives. For example: the reference time for the development of allergies or asthma for infants is not specified in this section, but later stated as being ages 4-6. It should be included here. Similarly for the “outgrowing asthma for asthma infants” (I understand this as meaning asthma exacerbation for asthmatic children). The first outcomes (development of disease) may be the result of exposure over the whole pregnancy period and the whole life of a child (a longer time effect), the latter (exacerbation of asthma) is probably the result of short term exposure. It seems that the outcomes to be studied for pregnant women are also possible responses to short term exposures, but these characteristics are not clear in the description.

Based on your comments, we included the statements in the objective (page 9 line 118-119) and outcome section (page 14 line 208-209, 211-213, 215-216). With respect to ‘outgrow’, it means the disappearance of symptoms for more than three years in the absence of medication. As pointed out, this was not explained in the manuscript. We hypothesized that desert dust exposure increases not only asthma incidence, but also non-outgrowers. Notwithstanding, based on your comments, we reconsidered whether we should include the analysis of outgrowers in this study, and decided against it. Although we still would like to address this question, our grant period is not sufficient to investigate outgrowers in detail. Thus, we deleted text related to outgrowers from the manuscript.

3. The second objective (“perform further analysis…”) is also not clear. One would say that it is about the effect modification potential of avoidance behaviors and vulnerability characteristics. This is not clear and it is further confused by the statement about “…what in the dust exerts its effects”, which prepares us for analyses of the chemical composition of dust, an issue not addressed in the rest of the paper.

This has been clarified in the objective (page 9 line 118-119).

4. Methods and analysis. The section entitled “setting”, tells extremely little about the setting, i.e. the three locations that will be studied. Are they urban? What is the population? Is there air pollution apart
from desert dust? Most important: is there particulate matter pollution? Is the “dust season” the same for all the areas? What is the usual PM concentration and how much is it during dust days?

Details were added to this section, as suggested (page 10 line 143-153).

5. Sample selection. The time frame is given but the characteristics of the enrolled pregnant women are not. At which stage of pregnancy are they? Will they have the possibility of being exposed during the subsequent dust season?

Typically, participants fill out consent forms during the second trimester of pregnancy, but we accepted enrolment just before delivery. The median duration between enrolment and expected delivery was 95 days (mean, 98 days; max, 218 days) (page 11 line 160-161). We can assess exposed/non-exposed status during pregnancy even for enrolled participants just before delivery, based on local dust measurements during pregnancy. However, it is impossible to assess avoidance behavior for these participants. Participants have the possibility of being exposed during the subsequent dust season, and we continue sending questionnaires on Asian dust days until the child reaches 4 years of age.

6. Study design. If the publication is going to be interesting for the research community, the full questionnaires should be included as an appendix. Table 1 aiming (I think) to provide information on the variables to be studied, is not informative, because it does not include the measurement of each variable. Additionally, how many control days per exposure day will be selected?

We included the full questionnaire as an appendix (page 34-47), and included additional information in Table 1 (page 31-32). The computer randomly selected participants for control days, with a probability of 10% every day during the Asian dust season. Accordingly, each participant typically gets 3 control days/month. On the other hand, the frequency of Asian dust days depends on the occurrence of a dust event in the desert and air mass movement from there, and therefore changes every season. The average frequency of Asian dust days defined by LIDAR >0.07/km is 10 days/year.

7. Sample size. For the primary purpose (asthma development- here referred as primary objective for the first time) the power calculations assume that there is a definition of low exposure levels, which does not appear in the paper. The second alleged power calculation (for worsening of allergic symptoms) is not presented at all in numbers.

An explanation was added regarding “lowly/highly exposed” (page 15 line 219-220, page 13 198-199). For the second purpose (short term effects on pregnant mothers), we obtained a sample size of 501 to detect an odds ratio of 1.5 (alpha=0.05, power=80%), assuming the occurrence of Asian dust to be 10 days and 20 control days for each pregnant mother, a response rate of 66%, and a probability of developing any symptom on control days of 5%. Because the sample size for infants is much larger, and the sample size calculation for pregnant mothers makes many assumptions, we simply indicate in the manuscript that ‘we confirmed this sample size to be sufficient’, for pregnant mothers.

8. Measurements. Demographics. More information is needed in this section and, particularly, which variables will be considered as confounders.

We added an explanation for measurements, demographics, and confounders or effect modifiers to the manuscript (page 13 line 198-199, page 15-16 line 226-242) and added Appendix in addition to
Table 1 (page 34-47).

9. Exposure. Here lies the main weakness of this paper. The description of the exposure is very important, as this is the novel characteristic of the adjunct study and relates to the main hypotheses. The description is completely inadequate. Choosing days with high concentrations of desert dust and defining these as exposure days and choosing control days (presumably with low levels of desert dust- this is not mentioned but may be inferred- , but within the dust season- are there really such days?) allows comparisons relevant to the short term health outcomes, such as worsening of symptoms in pregnant women or asthma exacerbation in an asthmatic child. Nothing is mentioned on the calculation of long-term exposure, which is necessary to assess the “primary outcome” (please see point 7 above). Clearly there is no definition of how this long-term exposure will be classified as low or otherwise.

We added the definition of Asian dust days and control days (page 13 line 198-199, page 15 line 223), and added an explanation for highly/lowly exposed (page 15 line 219-220). Asian dust clouds reach Japan mainly in the spring and fall, with some exceptions. Typically, increases in dust levels last one to two days. Multiple events can occur on occasion in a month, and it is also possible to have no events during a season.

10. Exposure. Is this method validated? Are there any measurements of particles in the study areas?

Suspended particulate matter (SPM) and PM2.5 are measured at each location, and we will conduct the same analysis for comparison. These measurements do not differentiate desert dust and other anthropogenic air pollutants, while LIDAR differentiates desert dust from others, although it does not differentiate particle size. As for validation, LIDAR measurements show a good correlation with mineral content in PM. We added more information (references) regarding the LIDAR measurements to the manuscript (reference 39-41).

11. Outcome. Please see point 6 above.

12. Confounders should be carefully described and they are not!

We added a description of confounders to the manuscript (page 15-16 line 226-242).

13. The data analysis methods are not mentioned at all, although they should be an integral part of a protocol.

We added an explanation of data analysis methods to the manuscript (page 14-16 line 217-242).

14. In the discussion, the “automatic random selection of questions” is not explained.

This expression was corrected (page 18 line 274-275).

15. The main discussion item, namely what are the potential confounders, including PM from other sources, is not touched. This needs to be addressed.
We added a discussion on this to the manuscript (page 17-18 line 263-273).

Minor comments.
The reference on which the authors are basing their statement that 40% of PM is from desert dust, is old and based on a model. Better evidence can probably be provided.
The paper will need English editing.

Thank you for your suggestion. We conducted literature searches and changed the descriptions and references. We also had the manuscript edited by a medical editor whose first language is English.

This version of the paper does not describe the intended statistical analyses. If there is a revised version than a statistical review may be necessary.

We added a statistical analysis section, as suggested (page 14-16 line 217-242).

Reviewer 2

This manuscript reported a protocol of ongoing birth cohort study in three regions of Japan to explore the effects of Asian dust exposure on children's asthma and allergic diseases. The study is adjunct with a large cohort study, The Japan Environment & Children's study (JECS), with a total of 100,000 children across 15 regions in Japan. The authors established online questionnaire delivery system to participant's mobile phones and/or personal computers on dust and non-dust days linked with the Light Detecting and Ranging (LiDAR) system which was used to define the dust days. The study enrolled 3,302 pregnant women by the end of 2012 and continues to enroll subjects up to March 2014.

Overall, the paper is well written and the design sounds relevant. The web-based questionnaire linked to the atmospheric monitoring system allowing for quick data collection can be a useful tool for researchers in this field. I have some minor issues to be raised.

Thank you very much for your kind comments.

1. Methods and analysis: It would be informative for readers if the authors could provide a data analysis plan in the paper. As summarized in Table 1, the study plans to collect many variables of individual characteristics, symptoms, behaviors etc. in a repeated manner for some of the variables, as shown in Figures 2 and 3, on dust and non-dust days. The readers may want to know how these variables can be incorporated in the analysis to answer three research questions on page 8. Specifically, the readers may be confused how the variables of the day-to-day symptoms and that for the allergic disease development can be combined or separate in the analysis.

Thank you for pointing this out. We added a data analysis plan, as suggested (page 14-16 line 217-242).

Minor comments

2. Title: As is guided in the journal instructions, I suggest to include the specific study type (e.g., cohort study) in the title.
The title was modified to indicate “a birth cohort” as suggested (page 1 line 1).

3. Setting: Although the authors provided references for the JECS design, some brief explanation of the eligibility criteria, if any, to participate the JECS would be helpful especially for readers who are unfamiliar with the Study.

We added a brief explanation for JECS including the eligibility criteria (page 10 line 136-140). Moreover, given that the study protocol paper of the JECS was recently published, we cited it in the manuscript (reference 36).

4. Measurements: line 52, page 11. Any reason to define the cutoff of the LIDAR desert dust measurement at 0.07/km? In addition, which altitude of the LIDAR measurement will be used? If it is based on the findings of the author’s previous study, probably the citation of the reference 17 here might be appropriate.

Because our preliminary results showed clear elevation of asthma hospitalization risk from around 0.07/km, and Ueda et al. also found elevation of ambulance dispatch risk above 0.07/km, we selected this as the cut-off value and have cited the relevant reference. We obtain LIDAR data at the 135 m level, as well as at the 135-1000 m level. We planned to use the 135 m value as the primary measurement, but found that the 135 m data in Toyama occasionally fails to differentiate between snow clouds and desert dust. Accordingly, we will also conduct the analysis using the 135-1000 m data. We added our definition of Asian dust days (page 13 line 198-200).

5. In the Table 1, “25(OH)D serum/immunoglobulin serum/inflammatory cytokines” is on the list and they seem to be measured by blood samples, but there is no description about it in the main text.

We added an explanation, as suggested (page 13 line 195-197).

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<th>REVIEWER</th>
<th>Klea Katsouyanni</th>
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<td>Department of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, Greece and Department of Primary Care &amp; Public Health Sciences and Environmental Research Group, King's College London, U.K.</td>
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**GENERAL COMMENTS**

Page 14, statistical analysis plan: The analysis plan itself is well written, while how the analysis plan corresponds to three objectives described in the end of the introduction is a bit unclear. I prefer matching the style of the Study design and Statistical analysis with that of the Objectives, for example, by creating items (i),(ii),(iii) in the Study design and Statistical analysis corresponding to each item in the Objectives. In addition, the third objective of “analysis to understand further the benefits and limitations of web-based questionnaires…” does not fit well with the other objectives because no clear methods and analysis are provided. Probably this would be the issue which can be incorporated in the discussion. To me, it would be more straightforward to rearrange the objectives like (i) to examine long-term effects, (ii) short-term effects, (iii) to examine the modifications of long- and short-term effects. However, this revision is not essential. It may not be fair to suggest what I did not do in the initial review, but I became more clearly understand the study objectives after reading the revised manuscript.

Page 13, line 198-200: The reference(s) are needed for the new sentence to validate the definition of the Asian dust day. In response to the authors’ comments, the reference 17 cited for the second sentence in the Exposure (i.e., the sentence starting “LIDAR is an optical….”) should be moved to right after the first (new) sentence (i.e., the sentence starting “Asian dust days were…”).

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**VERSION 2 – AUTHOR RESPONSE**

Reviewer: 2

1. Page 14, statistical analysis plan: The analysis plan itself is well written, while how the analysis plan corresponds to three objectives described in the end of the introduction is a bit unclear. I prefer matching the style of the Study design and Statistical analysis with that of the Objectives, for example, by creating items (i),(ii),(iii) in the Study design and Statistical analysis corresponding to each item in the Objectives. In addition, the third objective of “analysis to understand further the benefits and limitations of web-based questionnaires…” does not fit well with the other objectives because no clear methods and analysis are provided. Probably this would be the issue which can be incorporated in the discussion. To me, it would be more straightforward to rearrange the objectives like (i) to examine long-term effects, (ii) short-term effects, (iii) to examine the modifications of long- and short-term effects. However, this revision is not essential. It may not be fair to suggest what I did not do in the initial review, but I became more clearly understand the study objectives after reading the revised manuscript.

Thank you for proposing the better presentation. We completely agree with you and modified the presentation. (page 8 line 116-117, page 9 line 118-125, page 15 line 219, 222-223, 227-229)

3. Page 13, line 198-200: The reference(s) are needed for the new sentence to validate the definition of the Asian dust day. In response to the authors’ comments, the reference 17 cited for the second
sentence in the Exposure (i.e., the sentence starting “LIDAR is an optical....”) should be moved to right after the first (new) sentence (i.e., the sentence starting “Asian dust days were...”).

Thank you for the important point. We moved it. (page 13 line 201)

Reviewer: 1

The authors responded nicely to the comments and the paper has been greatly improved.

Thank you for your kind comments.
The improvements owe you very much.

I have three remaining comments:
1. In the Abstract it must be mentioned that the purpose of this paper is to present the study protocol.

We added it in the abstract. (page 3 line 32)

2. The statistical analysis section must be somewhat improved: when the analysis is about the time to event, aren't Cox' proportional hazards models going to be applied?

Yes, we are. We added it in the statistical analysis section. (page 15 222-223)

3. In Table 1, in the footnote JECS must be written in full and the questionnaire in the appendix also mentioned for further reference. Quantitative variables in the Table could have their units mentioned.

We added the full description for JECS and units for variables, thank you. (page 33 line 474)
Birth cohort study on the effects of desert dust exposure on children's health: protocol of an adjunct study of the Japan Environment & Children's Study

Kumiko T Kanatani, Yuichi Adachi, Nobuo Sugimoto, Hisashi Noma, Kazunari Onishi, Kei Hamazaki, Yoshimitsu Takahashi, Isao Ito, Miho Egawa, Keiko Sato, Tohshin Go, Youichi Kurozawa, Hidekuni Inadera, Ikuo Konishi, Takeo Nakayama and Japan Environment & Children's Study Group

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