PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	THE PEBBLES STUDY PROTOCOL: A randomised controlled trial to prevent atopic dermatitis, food allergy and sensitisation in infants with a family history of allergic disease using a skin barrier improvement strategy
AUTHORS	Lowe, Adrian; Su, John; Tang, Mimi; Lodge, Caroline J.; Matheson, Melanie; Allen, Katrina; Varigos, George; Sasi, Arun; Cranswick, Noel; Hamilton, Simone; Robertson, Colin; Hui, Jennie; Abramson, Michael; O'Brien, Shaie; Dharmage, Shyamali

VERSION 1 – REVIEW

REVIEWER	Cristina Lopes Medical Faculty Porto University Portugal Allergy Unit Pedro Hispano Hospital Matosinhos Portugal
REVIEW RETURNED	28-Jun-2018

GENERAL COMMENTS	I would like to see the appendices that i think were not provided Since food allergy is a primary objective I think the authors should consider evaluating also other relevant allergens to a specific child than milk, egg and peanut if the parents reported a clinical suggestive history for example to fish or wheat. Authors should also consider describing in more detail food challenges procedures: what are the criteria of positivity -do authors consider the diagnosis of food allergy only IgE mediated allergy with symptoms of urticaria, wheezing, anaphylactic reactions or also when occurring a delayed exacerbation of AD 48 to 72 after the oral food challenge? If yes the authors should consider examining the child after this period and determining the increment of EASI that they would consider clinically relevant.

REVIEWER	Prof. Dr. med. Margitta Worm Charité-Universitätsmedizin Berlin Germany
REVIEW RETURNED	04-Jul-2018

GENERAL COMMENTS	- data from this study has been published this year (Lowe et al., BJD, 2018) - It is required to indicate this and to mention whether the data collection has been completed yet? - Limitation: 1. No placebo emollient group – no control of self-use 2. Is IgE sensitization determined in sIgE detection as well 3. Is statistical power sufficient to detect the role of filaggrin deficiendy - Bias by randomization of high risk infants (impact mother-father may differ as well) - How is food allergen exposure controlled (new guidelines)

	- Missing as an assessment “onset of sensitization against EpiCeram” Comments to the Editor: can the study protocoll still be published according to the Guidelines of BMJ-open?

REVIEWER	Sandipan Dhar Institute of Child Health Kolkata India
REVIEW RETURNED	22-Sep-2018

GENERAL COMMENTS	It is a brilliant and up-to-date research.You have addressed the most important issue related to Atopic Dermatitis prevention in the current days. You have done it it in a very meticulous and scientific way with lot of sincerity, dedication and honesty. I appreciate it a lot ! However, for understanding of all those who do not work in the field, some areas need elaboration. The strength of your study is its originality, large sample size and correct methodology.There are minor lacunae which need to be addressed. I am sure it will add a great value to our understanding of AD in current days' perspective.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1 - Cristina Lopes

Comment 1: I would like to see the appendices that i think were not provided

Response: We have attached the appendices for the reviewer’s information.

Comment 2: Since food allergy is a primary objective I think the authors should consider evaluating also other relevant allergens to a specific child than milk, egg and peanut if the parents reported a clinical suggestive history for example to fish or wheat.

Response: We have a limited budget to conduct this study, so we have elected to restrict the number of food allergens screened (by SPT) and confirmed by oral food challenge in this study to egg, milk and peanut, as they are the major food allergens in Australian infants(1). Other allergens, such as sesame, fish and wheat generally have low prevalence (<0.5%)(2). Most parents (<20%) in this setting do not expose their child to tree nuts in the first year of life and reported reactions are very rare (0.1%)(3). However, if children develop symptoms that are consistent with food allergy, we will direct participants to seek clinical evaluation, which is available to all Australians. If a clinical diagnosis of allergy to another food allergen is made, then we will capture this information during our survey and it will form a secondary outcome. We have added this as a secondary outcome to the manuscript.

Comment 3. Authors should also consider describing in more detail food challenges procedures: what are the criteria of positivity -do authors consider the diagnosis of food allergy only IgE mediated allergy with symptoms of urticaria, wheezing, anaphylactic reactions or also when occurring a delayed exacerbation of AD 48 to 72 after the oral food challenge? If yes the authors should consider examining the child after this period and determining the increment of EASI that they would consider clinically relevant.

Response: In this study, we will use objective, predefined, stopping criteria for oral food challenges (OFC) according to standardised stopping criteria guided by PRACTALL (4). Specifically, a positive OFC result will be defined as any of the following signs occurring within 2 hours of ingestion:

- 3 or more concurrent noncontact urticaria;

- Generalized marked erythema;
- Continuous rubbing of nose/eyes, periocular swelling or long bursts of sneezing, persistent rhinorrhoea.
- Wheeze, use of accessory muscles
- Hoarseness, frequent dry cough or stridor
- 2 or more events of emesis or diarrhoea
- evidence of circulatory/neurologic compromise (>20% drop in blood pressure from baseline or change in mental status, collapse or impaired circulation).

If a child passes the oral food challenge at the clinic visit, parents will be asked to continue feeding that food from day 2 to 7. A positive challenge will also be considered if any of the above reactions occur within 2 hours of ingestion of the food during this period at home. As such, an isolated flare of AD is not sufficient to be identified as a positive OFC.

Reviewer: 2 - Prof. Dr. med. Margitta Worm
Comment 1- data from this study has been published this year (Lowe et al., BJD, 2018). - It is required to indicate this and to mention whether the data collection has been completed yet?

Response: We published the pilot study work earlier this year and cite this study as reference 13. This proof of concept study was a randomised trial of this intervention with 80 infants. The current protocol paper describes our new study of 760 infants. While the intervention remains the same as the previous study, our protocol has extended to include food challenges to allow confirmed food allergy to be measured as an outcome, and collection of biological samples. We have revised the wording of the introduction to make this clearer, including mentioning that it was our PEBBLES pilot study, and adding the following sentence to the introduction.

"This study follows on from our published pilot study(5). "

Comment 2- No placebo emollient group – no control of self-use

Response: We have elected to not use a placebo emollient in this trial. It remains to be determined if this form of intervention prevents food allergy or has longer term (post intervention) effects on eczema outcomes. There is some evidence that standard emollients reduce the risk of eczema. As the current standard of care does not include routine prophylactic emollient use for the purposes of prevention, a head-to-head comparison of two emollients would not be relevant, and risks showing null results as both emollients may have some degree of efficacy. We have designed this study to test the potential impact of this form of intervention, using the most intensive intervention tested to date (twice daily allocation EpiCeram) to demonstrate efficacy. If this study is positive, further comparison with other study results, or new head-to-head studies of different emollients will be needed to determine the relative effectiveness of alternate interventions.

Comment 3. Is IgE sensitization determined in sIgE detection as well

Response: We do not intend to collect data on sIgE in this study for logistical reasons.

Comment 4. Is statistical power sufficient to detect the role of filaggrin deficiendy

Response: As noted in the manuscript, we will be collecting check swabs from infants and genotyping for filaggrin null mutations. We do not aim to measure the effect of filaggrin null mutations on allergic disease outcomes, as these have been previously demonstrated. However, we will test if the intervention is effective in those participants with one or more filaggrin null mutation. We have added the following to the sample size section.

"As the population prevalence of FLG null mutations is approximately 10% [16], and we will only recruit children with a family history of allergic disease, we expect at least 15% of participants to have one or more FLG null mutations. This will result in 45 infants per group (allowing for up to 80% loss to follow-up) with one or more such mutations. We will have approximately 80% power to detect a relative risk of 0.40 (20% vs 50%) induced by the treatment for the outcome of AD."

Comment 5. Bias by randomization of high risk infants (impact mother-father may differ as well)

Response: Randomisation of high-risk infants will not bias the findings of our study, but it will impact on the transferability of results, so we would not generalise the findings to the low risk population (no family history of allergic disease). We agree that maternal versus paternal allergic diseases may impact differentially on allergic disease risk in the infant. Stratification by number of affected family members will ensure balance for this factor. Randomisation will ensure that any differences between the intervention and control groups are random, and differences are likely to be minor with the sample size that will be recruited.

Comment 6. How is food allergen exposure controlled (new guidelines)

Response: We have added the following to the methods section

"All participants (in both intervention and control groups) will be provided with the Australasian Society of Clinical Immunology and Allergy's advice on strategies for allergy prevention (https://www.allergy.org.au/patients/allergy-prevention/ascia-guidelines-for-infant-feeding-and-allergy-prevention). This advice, updated in 2016, recommends "introduction of solid foods around 6 months, but not before 4 months" and that "introduction of common allergenic foods should not be delayed". In addition, we will send participants a reminder of this advice at 6 months of age, and monitor introduction of foods into the infant diet as part of the weekly diary cards."

Comment 7. Missing as an assessment "onset of sensitization against EpiCeram"

We have added the following as a secondary outcome

"Parents will be asked to immediately contact the study team if they believe that their child has developed an adverse reaction to the EpiCeram™ treatment. We will instruct parents to cease application of the treatment immediately and wash their infants skin with water. We will organise an assessment with a study dermatologist. Parents will be asked to apply a small amount to the child's forearm on the day prior to the appointment. Reactions to EpiCeram will be confirmed if repeatable symptoms are caused by EpiCeram application."

Reviewer: 3
Reviewer Name: Sandipan Dhar
Institution and Country: Institute of Child Health, Kolkata, India
Please state any competing interests or state 'None declared': NIL

Please leave your comments for the authors below
Comment 1: It is a brilliant and up-to-date research.You have addressed the most important issue related to Atopic Dermatitis prevention in the current days. You have done it it in a very meticulous and scientific way with lot of sincerity, dedication and honesty. I appreciate it a lot !
However, for understanding of all those who do not work in the field, some areas need elaboration.

The strength of your study is its originality, large sample size and correct methodology.There are minor lacunae which need to be addressed.
I am sure it will add a great value to our understanding of AD in current days' perspective.
Response: Thank you for these positive comments.

Comments from the manuscript

Comment 1: In fact there is one more work towards this goal which needs to be acknowledged : "https://www.ncbi.nlm.nih.gov/pubmed"PLoS One. 2018 Feb 28;13(2):e0192443. doi: 10.1371/journal.pone.0192443. eCollection
Response: This paper(6) is a sub study nested within one (7) of the three clinical trials on this topic published to date. The aim of this study was to examine if routine emollient use impacted on skin biome, pH and water capacitance at six months of age.

We have added the following statement in reference to this study. "We will also explore if any preventive effects are mediated either by alteration of skin lipid or microbiome, as there is some evidence that routine emollient use may alter skin biome(6)."

FORMATTING AMENDMENTS (if any)
Required amendments will be listed here; please include these changes in your revised version:
Comment: Please remove all your figures in your main document and upload each of them separately under file designation 'Image' (except tables and please ensure that Figures are of better quality or not pix-elated when zoom in). NOTE: They can be in TIFF or JPG format and make sure that they have a resolution of at least 300 dpi and at least 90mm x 90mm of width. Figures in PDF, DOCUMENT, EXCEL and POWER POINT format are not acceptable.
Response: We have made these changes to the manuscript files.

Comment: We have noticed that you have uploaded the file "PEBBLES protocol_v1.4_171102_clean" under 'supplementary file'. However, we can't see any citation for this file within the main text. If this file needs to be published as supplementary file, please cite it as 'supplementary file' in the main text. Otherwise, kindly change its file designation to 'Supplementary file for editors only'.
Response: We have changed the file designation to "supplementary file for editors only"

Comment:- We have implemented an additional requirement to all articles to include 'Patient and Public Involvement' statement within the main text of your main document. Please refer below for more information regarding this new instruction:
Authors must include a statement in the methods section of the manuscript under the sub-heading 'Patient and Public Involvement'.
This should provide a brief response to the following questions:
How was the development of the research question and outcome measures informed by patients' priorities, experience, and preferences?
How did you involve patients in the design of this study?
Were patients involved in the recruitment to and conduct of the study?
How will the results be disseminated to study participants?
For randomised controlled trials, was the burden of the intervention assessed by patients

themselves?
Patient advisers should also be thanked in the contributorship statement/acknowledgements.
If patients and or public were not involved please state this.

Response: We have added the following section to the manuscript.

'Patient and Public Involvement'. "Patients and the public were not involved in the development of this study protocol. All participants will be sent an annual study update newsletter and a summary of the study results at the completion of the trial."

References

1. Osborne NJ, Koplin JJ, Martin PE*, et al.* Prevalence of challenge-proven IgE-mediated food allergy using population-based sampling and predetermined challenge criteria in infants. *J Allergy Clin Immunol* 2011;**127**:668-76 e1-2.
2. Perkin MR, Logan K, Tseng A*, et al.* Randomized Trial of Introduction of Allergenic Foods in Breast-Fed Infants. *N Engl J Med* 2016;**374**:1733-43. doi:10.1056/NEJMoa1514210
3. McWilliam V, Peters R, Tang MLK*, et al.* Patterns of tree nut sensitization and allergy in the first 6 years of life in a population-based cohort. *J Allergy Clin Immunol* 2018. doi:10.1016/j.jaci.2018.07.038
4. Sampson HA, Gerth van Wijk R, Bindslev-Jensen C*, et al.* Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology-European Academy of Allergy and Clinical Immunology PRACTALL consensus report. *J Allergy Clin Immunol* 2012;**130**:1260-74. doi:10.1016/j.jaci.2012.10.017
5. Lowe AJ, Su JC, Allen KJ*, et al.* A randomised trial of a barrier lipid replacement strategy for the prevention of atopic dermatitis and allergic sensitisation: The PEBBLES Pilot Study. *Br J Dermatol* 2018;**178**:e19-e21. doi:10.1111/bjd.15747
6. Glatz M, Jo JH, Kennedy EA*, et al.* Emollient use alters skin barrier and microbes in infants at risk for developing atopic dermatitis. *PLoS One* 2018;**13**:e0192443. doi:10.1371/journal.pone.0192443
7. Simpson EL, Chalmers JR, Hanifin JM*, et al.* Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention. *J Allergy Clin Immunol* 2014;**134**:818-23. doi:10.1016/j.jaci.2014.08.005

VERSION 2 – REVIEW

REVIEWER	Cristina Lopes, MD , PhD Medical Faculty Porto University Portugal Allergy Unit Pedro Hispano Hospital Matosinhos Portugal
REVIEW RETURNED	08-Dec-2018

GENERAL COMMENTS	The authors should consider using the universally accepted diagnostic criteria for AD of Hanifin JM Rajka G Diagnostic features of atopic dermatitis. Acta Dermatol Venereol. 1980; 92 : 44-47 instead of the U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis