BMJ Open

Preventive Antenatal Educational Program on Allergic Diseases (PAEPAD) versus standard antenatal care for prevention of atopic dermatitis: study protocol for a single-center, investigator blinded randomized controlled trial.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-048083
Article Type:	Protocol
Date Submitted by the Author:	22-Feb-2021
Complete List of Authors:	zhao, mutong; Beijing Children's Hospital Capital Medical University, Dermatology Liang, Yuan; Beijing Children's Hospital Capital Medical University, Dermatology Song, Fengli; Capital Medical University Daxing Teaching Hospital Ma, Lili; Capital Medical University Daxing Teaching Hospital Wang, Ying; Capital Medical University Daxing Teaching Hospital Gao, Wanli; Capital Medical University Daxing Teaching Hospital Tian, Jing; Beijing Children's Hospital Capital Medical University, Dermatology Ying, Xiangji; Peking University Cancer Hospital Shen, Chunping; Beijing Children's Hospital Capital Medical University Wang, Shan; Beijing Children's Hospital Capital Medical University Jiao, Lei; Beijing Children's Hospital Capital Medical University Wang, Yang; Beijing Children's Hospital Capital Medical University Sun, Xiaoyan; Capital Medical University Daxing Teaching Hospital Ma, Lin; Beijing Children's Hospital Capital Medical University, Dermatology Ma, Xiuhua; Capital Medical University Daxing Teaching Hospital
Primary Subject Heading:	
Secondary Subject Heading:	
Keywords:	Eczema < DERMATOLOGY, EPIDEMIOLOGY, PAEDIATRICS, Paediatric dermatology < PAEDIATRICS

TITLE PAGE

Preventive Antenatal Educational Program on Allergic Diseases (PAEPAD) versus standard antenatal care for prevention of atopic dermatitis: study protocol for a single-center, investigator-blinded randomized controlled trial.

Mutong Zhao[1], Yuan Liang[1], Fengli Song[2], Lili Ma[2], Ying Wang[2], Wanli Gao[2], Jing Tian[1], Xiangji Ying[3], Chunping Shen[1], Shan Wang[1], Lei Jiao[1], Yang Wang[1], Xiaoyan Sun[4], Lin Ma[1], Xiuhua Ma[2]

1 Department of Dermatology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China, 100045.

2 Department of Obstetrics and Gynecology, Beijing Daxing District People's Hospital-Capital Medical University Daxing Teaching Hospital, Beijing, China, 102600.

3 Gastrointestinal Cancer Center, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Peking University Cancer Hospital and Institute, Hai-Dian District, Beijing, China, 100142

4 Department of Dermatology and Venerology, Beijing Daxing District People's Hospital-Capital Medical University Daxing Teaching Hospital, Beijing, China, 102600.

Correspondence to:

Lin Ma, MD, Department of Dermatology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China. E-mail: bch_maleen@aliyun.com. Tel: +86 13601305676

Xiuhua Ma, MD, Department of Obstetrics and Gynecology, Beijing Daxing District People's Hospital, Capital Medical University Daxing Teaching Hospital, Beijing, China. E-mail: mxhdxqyy@126.com Tel: +86 13381021859

Word count: 4407

ABSTRACT

Introduction

Patient education serves an essential purpose in the long-term management of allergic diseases as a secondary prevention approach. However, evidence on using education for primary prevention is limited. This study aims to evaluate the effect of an educational intervention, i.e., the Preventive Antenatal Education Program on Allergic Diseases (PAEPAD), on infantile allergic disease incidences.

Methods and analysis

This is a single-center randomized controlled trial of expecting mother-children dyads in Daxing Teaching hospital of Beijing, China. A total of 2266 expecting mothers will be recruited. Expecting mothers enlisted in the birth registry of Daxing Teaching Hospital of Capital Medical University and intend to give birth at this location will be screened for eligibility. Women at high risk for miscarriage or intend to have abortions will be excluded. The participants will be allocated into two groups (i.e., the PAEPAD and the standard care group) by random allocation (1:1). The PAEPAD group will receive a multi-disciplinary education of neonatal care, whereas the standard care group will receive the standard neonatal care education carried out by obstetricians. They will be followed for two years. The primary outcome will be infantile atopic dermatitis (AD) cumulative incidence at two years post-partum. Secondary outcomes will include other AD outcomes, atopic march outcomes, knowledge outcomes, and other maternal and neonatal outcomes. Data collection will be carried out using both electronic and paper questionnaires. Biological samples will also be collected longitudinally.

Ethics and dissemination

The study design was approved by the ethical committee of Capital Medical University Daxing Teaching Hospital, Beijing, China. The trial results will be published in peer-reviewed journals and at conferences.

Trial registration

The trial is prospectively registered at the ChiCTR registry (Trial ID: ChiCTR2000040463).

Strengths and limitations of the study

Large single-center investigator-blinded randomized controlled trial (RCT).

First attempt to investigate education for primary prevention of incident cases of allergic diseases (atopic dermatitis for primary outcome).

Prone to potential contamination due to single-center design and non-medication intervention.

Potential heterogeneity of intervention effect due to variation in how the intervention will be carried out by individual educator albeit provision of formal training for educators.

KEYWORDS

Primary prevention, atopic dermatitis, atopic march, health education, antenatal education, pediatric, study protocol

INTRODUCTION

Atopic disorders place a substantial burden on both individuals and the health care system[1-4]. The sequential occurrence of AD, followed by one or more disorders characterized by allergen-specific type 2 (including TH2) responses are designated as atopic march[5]. The underlying mechanisms feature both a genetic susceptibility in which barrier dysfunction and immune dysregulation predispose atopic individuals to Th2 immunity, and a progression in which AD and its pathological changes function as instigating events to subsequent development of other atopic comorbidities[5 6]. The human skin is a functional immune organ with an abundance of immunocompetent cells. In the resting state, an intact skin barrier and a balance of immune cell populations, cytokines, and chemokines promote immune tolerance, which is otherwise disrupted in AD patients. Barrier dysfunction from both loss-of-function mutations in filaggrin and the itch-scratch cycle was postulated as the driving component for atopic march programming by promoting Th2 and Th17 differentiation[7-9]. Subsequently, sensitizations to food allergens develop in the setting of Th2 skewing, which is otherwise Th1 skewed in the gastrointestinal-homing T cells in tolerant subjects[10]. Similarly, in asthmatic airways, disrupted junctional adhesion, mucus plugging develops due to Th2 polarization. While the severity of subsequent allergic airway inflammation was affected by AD, abrogation of AD by topical treatment prevents worsening of subsequent airway inflammation by counteracting the Th17 pathway [11]. Granted that both the genetic predisposition and the atopic progression contribute to the development of atopic march, longitudinal studies are warranted to explore the impact of immune activation of AD on the development of other

TH2 comorbidities. Meanwhile, the role of epithelial-immune crosstalk in the atopic march needs to be further elucidated.

To date, extensive research has been conducted on the therapeutic effect of barrier enhancement using emollients. Compelling evidence indicated that emollients render the skin less susceptible to irritants and reduce flares of AD (secondary prevention)[12] and therefore was regarded as a cornerstone of AD therapy[13]. However, evidence was inconsistent regarding the preventive effect of emollient application [12 14-17]. The discordance from these behavioral intervention studies is probably a result of the difference in when the outcome assessment took place. In the BEEP[12] and PreventADALL[14] studies, the outcome was measured after a long washout period to ensure that any mild AD would not be concealed by the on-going application of emollients, whereas in the small studies [15 16] the immediate effect was evaluated. However, the null long-term result should not overshadow the strong efficacy signal displayed at the immediate outcome assessments. Moreover, as behavior change is a process that unfolds through a continuum of stages from pre-contemplation, contemplation, preparation, to action and maintenance, such straightforward behavioral intervention may not echo with the real-world scenario, where behavior changes are achieved through health promotion initiatives, including health education and health policies [18 19]. Consequently, the generalizability of these studies might be affected due to the difference in how intervention was delivered and the setting under which outcome assessment took place.

In realizing the tremendous burden inflicted by atopic disorders and the critical role of AD in atopic march, significant therapeutic discoveries have been made over the past century. Nonetheless, in AD and other chronic diseases alike, adherence to treatment can be strikingly poor [20]. The underlying factors were postulated to be the complexity of treatment regimen, corticosteroid phobia, and caregiver burden [20]. Addressing these issues calls for consistent efforts in patient education that can hardly be accomplished in a typical clinical visit. Recent studies have shown that structured patient education can significantly reduce disease severity in AD patients [21]. Given this, guidelines for AD have acknowledged education of patients and caregivers as an essential form of secondary prevention to reduce disease flares and improve quality of lives[22]. However, little is

known about how education performs as a primary prevention approach to reduce AD disease burden.

Antenatal education is a crucial component of antenatal care. Since its implementation from the nineteenth century, mortality in children has decreased tremendously. In 2005, the World Health Organization called for "realizing the Potential of Antenatal Care"[23], and thence started using it as a platform for primary prevention of malnutrition, HIV/AIDS, sexually transmitted infections, tuberculosis, and prevention of postpartum and neonatal diseases [24-26]. Its diversified usage has led to us hypothesize that educational intervention delivered through the antenatal education platform may yield informative data for assessing the primary prevention of allergic diseases. Thus, the Preventive Antenatal Education Program on Allergic Diseases (PAEPAD) study focuses on using the antenatal care platform as a more affordable and effective health education approach. In this investigator-blinded, randomized controlled trial, we aim to evaluate the effect of such intervention vs. standard education on atopic disease outcomes. The primary objective is to evaluate the preventive effect of PAEPAD on atopic dermatitis and atopic comorbidity incidences. The secondary objectives are: 1) to explore the immune-barrier crosstalk between the immune system, commensal flora and skin barrier function that may explain the development of atopic march; 2) to identify biomarkers (metabolites, MicroRNAs) that can be used to characterize individuals at high risk of AD and atopic march; 3) to elucidate whether and to what extent the maternal immune milieu influences AD development of the child through cord blood and breast milk.

METHODS AND ANALYSIS

Study design

The study is an exploratory prospective investigator-blinded randomized controlled trial with two arms (PAEPAD vs. standard antenatal education). All expectant mothers planning to give birth at the Daxing Teaching Hospital of Capital Medical University will be invited to participate. Daxing Teaching Hospital of Capital Medical University is a general hospital located in Daxing district of Beijing, China, with more than 6,000 deliveries annually. Daxing district makes up approximately 6.3% of Beijing geographically. Daxing Teaching Hospital of Capital Medical University makes up

approximately 2.7% of Beijing's annual deliveries. This protocol was drafted (Aug 17th, 2020) before participant recruitment (Oct 30th, 2020).

RECRUITMENT, INCLUSION AND EXCLUSION CRITERIA

All expectant parents living in the catchment area of the Daxing Teaching Hospital of Capital Medical University will receive an information leaflet about the study at their first visit to the maternity clinic. Recruitment will take place in their first mandatory maternity class between gestation week 7 to 14+6. All expectant mothers will be given detailed information about the study. A trained research nurse will outline key information (i.e., inclusion and exclusion criteria, scheduled research visits, and instructions on biological sample collection) about the study in the form of a short lecture session. The parents who wish to participate will be requested to provide written informed consent. They will be inquired on five levels of consent: 1) consent to participate in the study; 2) consent to biological sample collections of the mother that are non-invasive, including but not limited to the recollection of blood from routine pregnancy workups; 3) consent to biological sample collections of the mother that are minimally invasive or could potentially cause discomfort, i.e., additional blood draw and vaginal swabs; 4) consent to biological sample collections of the child that are non-invasive, including but not limited to the recollection of blood from routine checkups; 5) consent to biological sample collections of the child that are minimally invasive or could potentially cause discomfort, i.e., additional blood draw and tape stripping. At birth, another consent form on the children's biological sample collection will be signed to allow for any change of consent status on non-invasive and minimally invasive sample collections. Consented participants will subsequently receive a QR code through their cellphone after registration. By scanning the QR code, the recruitment staff will be able to confirm their participation status, at the end of which a study-specific ID number will be generated automatically.

Inclusion and exclusion criteria

Run-in phase inclusion criteria: 1. Enlisted in the birth registry of Daxing Teaching Hospital of Capital Medical University and intend to give birth at this location; 2. Women aged ≥18; 3. Less than 14+6 gestational week when recruited as measured by last

menstrual period; 4. Residents of Daxing and intend to remain residing in Daxing for at least two years postpartum; 5. Written consent form.

Randomization phase inclusion criteria:

Pregnant women enlisted in the birth registry of Daxing Teaching Hospital of Capital Medical University and intend to give birth at this location; passes the run-in phase criteria.

Withdrawal criteria: 1. Still birth; 2. Abortion (spontaneous or induced); 3. Rare comorbidities that present after inclusion into the study that may render the participant unsuitable for participation, including but not limited to malignancies, amniotic fluid embolism, eclampsia, and major birth abnormalities of the child.

Exclusion criteria: 1. Planned abortion; 2. Rare comorbidities of the mother that may cause miscarriage and congenital disabilities as determined by OBs, including but not limited to malignancies, congenital heart diseases, monogenetic diseases; 3. Recurrent miscarriage; 4. Mental, psychological or intellectual disabilities of either one of the expecting parents.

Treatment allocation

The study flow is as follows (Fig. 1): Participants will be randomly assigned to one of the two arms (i.e., PAEPAD vs. standard antenatal care). Individual randomization will be conducted by an epidemiologist using a computer-generated list with the number of groups being 2 and the distribution ratio of the two groups being 1:1. The list will be generated using block randomization with block sizes hidden from all investigators. Group allocation will be placed in sealed opaque envelopes, labeled by numbers only. The envelopes will be opened in consecutive order. Participants will then be informed about their allocated groups.

Blinding

This is a researcher-blinded study. Treatment allocation will be performed by an epidemiologist, and the researchers who evaluate the outcome matrices and analyze data will work independently from the group of clinicians who will carry out the intervention. Data entry will be undertaken by trial administrators blinded to group allocation.

Intervention

After randomization at GW 30, the participants will be informed on their allocation, and members of the research team will send out weekly invitations through E-mail to participants who have not yet completed the intervention. For those who failed to complete the intervention prior to admission into the OB department, a pre-recorded video will be played during their hospital stay. In the standard care group, patients will receive the standard neonatal care session from an experienced obstetrician, which will include breastfeeding, newborn screening tests, infant physiology, immunization, solid food introduction, belly and eye care (45 min). This session is one of the five mandatory sessions with participation of 83.7-91.9% over the past five years (unpublished data). The treatment group will receive an educational program designed by a multi-disciplinary group of experienced obstetricians and pediatric dermatologists. The program will be focused primarily on: 1) Standard education of neonatal care as the control group (45 min); 2) Skin care of the newborns with a practical demonstration on bathing and emollient application (20 min); 3) Sun protection (3 min); 4) A brief introduction on commonly used topical agents during infancy, including topical corticosteroids, antibiotics, and astringents (5 min); 5) Besides, the program will also contain a 5 minutes presentation on atopic dermatitis disease burden, its precipitators, managements, disease courses, and the atopic march. At the neonatal care class, all participants will first receive the standard education, which will be held concurrently in two separate rooms to minimize group contamination. At the end of the sessions, participants of the PAEPAD cohort will be asked to participate in the PAEPAD session, which will last for less than 40 min. And participants of the standard neonatal care cohort will be asked to leave. Any crossover and non-compliance will be surveyed by a research nurse at the beginning and end of the antenatal sessions. The research nurses who collect data on compliance and who send out invitations will not be involved in outcome assessment.

Study outcomes and follow-up

Study outcomes

All outcome measures are summarized in Table 1. There will be both fixed and disease prompted postnatal follow-up time points during which outcome assessments will take place.

Diagnostic criteria for AD will be based on Hanifin&Rajka criteria, which is regarded as the 'gold standard' for hospital-based research. The Infants' Dermatitis Quality of Life Index (IDQoL) is a questionnaire of ten items that has been translated into 21 languages. This questionnaire is validated in infants aged 0–3 years[27]. Disease severity at disease flares will be measured by SCORing of Atopic Dermatitis (SCORAD) and Eczema Area and Severity Index (EASI) and through the course of the disease by Investigator's Global Assessment (IGA). SCORAD and EASI are validated instruments for disease severity assessment [28]. IGA provides the most straightforward assessment of disease severity and will thus be employed to assess AD disease-free days. An IGA of less than two is defined as clearance of disease. An episode of expiratory wheezing will be defined as bronchial obstruction lasting for at least 24 hours preceded by at least a one-week non-wheezing healthy period, as defined by a physician. Recurrent wheezing will be defined as the occurrence of 3 or more episodes of expiratory wheezing diagnosed by a physician in a 12-month period[29]. Rhinitis will be defined as symptoms of sneezing, a runny or blocked nose, or itchy, red and watery eyes after exposure to furred pets or pollen the year before follow-up and/or doctor's diagnosis of allergic rhinitis[30]. Sensitization will be defined as allergen-specific IgE ≥0.35 kU_A/l. Parental knowledge and attitude will be quantified using a questionnaire. The items of the questionnaire were developed following rigorous procedures, including a review of literature, a patient focus group and a panel discussion of experts. This questionnaire is currently under validation (unpublished data).

Follow-up

Participants will receive both fixed and disease prompted follow-up visits. Follow-up visits will be carried out at Daxing hospital and five health service centers during immunization. At follow-ups, biological samples, surveys and physical examination data will be collected. Specific items are listed in Table. 2. In addition, children will be assessed upon disease onsets and flares. During follow-ups, all cases will be treated according to the guidelines [2 13 31-34] by specialists who are actively engaged in the care of pediatric AD patients. Participants are not allowed to participate in other clinical trials after inclusion into the study till the end of the last follow-up visit

Covariates

Relevant covariates will include age, sex, social-economic status, familial history of allergic diseases, administration of systemic medication and nutrient supplements, maternal psychological status measured with Kessler-10 prenatally. Other variables, including the Edinburgh postnatal depression scale (EPDS), maternal comorbidities during pregnancy, postnatal nutrition status and indicators for feeding practices (i.e., minimum dietary diversity, the introduction of solid, semi-solid or soft foods, duration of breastfeeding) will also be collected.

Data collection and management

Data collection at fixed time points will be conducted with an electronic database designed specifically for this project. At each visit, patients will be asked to present a patient-specific QR code, by scanning which two different questionnaires will be delivered to the participants and a research nurse separately. Less than 15 items will be surveyed in a standard questionnaire for the patient to minimize respondent fatigue caused by lengthy questionnaires. The rest of the relevant data will be collected by the research nurses during a face-to-face interview. Routine lab workups will be collected from the participants' medical records. Data will be checked by the members of the research team, and incorrect or missing questions will be sent back to the participants. All data recorded in this electronic database will be accessible only by the team members. Participants will receive text reminders prior to each follow-up visit and will be interviewed through phone calls for incomplete visits. Data collection at disease onsets and flares will be carried out with paper surveys, which will be encrypted and kept accessible only by team members with authorization. Group allocation data are accessible through unique identifiers on a separate sheet by only the principal investigators. All data handling (data entry, storage, and analysis) will be confidential. The principal investigators are responsible for ensuring data quality.

Biological sample collection

At each time point, biological samples from mothers and children (where applies) will be collected and stored (Table 2). These samples include maternal blood, urine, feces, skin, vaginal and oral swabs, breast milk, placenta, cord blood, meconium, and blood, feces,

skin, nasopharyngeal and external auditory canal swabs, tape stripping of skin lipids from the children. Procedures are detailed in the supplementary material.

Sample size

The PAEPAD study will be based on a sample of 2266 expecting mothers. We calculated that assuming 20% [35] AD rate, 20% lost to follow up (LTFU), with a clinically significant estimate of a cumulative incidence ratio of 0.75, 80% power and two-sided 5% significance level, the estimated sample size of the primary outcome would be 2266. The cutoff value of clinical significance of such educational intervention was derived by a survey of expert opinion (n=7, unpublished data) and from previous RCTs with behavioral interventions [15 17].

Analyses

Definition of population sets

1) Primary analysis population: The Modified Intent-to-treat Population (MITTP), which will comprise of expectant women who undergo randomization, with data of at least one post-intervention follow-up.
2) Per-protocol Population (PPP): All expectant women complying with the study protocol, with data of at least one post-intervention follow-up.
3) As treated population: All randomized participants who received the intervention (whether complied or not) , with data of at least one post-intervention follow-up.

Statistical analysis

Statistical analyses will be performed using STATA 14.0, R 1.0.44 and SAS9.2 statistical software.

The primary analysis will be based on the MITT population. Sensitivity analyses will be done with both the PP population and the as-treated population. For primary analyses, we will use χ^2 tests to compare categorical outcomes and present risk ratios and risk differences with 95% confidence intervals. For continuous variables, normally distributed continuous variables will be compared using the t-test, and the Wilcoxon rank-sum test will be used for skewed variables. For time to event data, e.g., time to first AD episode and time to the first topical corticosteroid exposure, will be calculated using the Kaplan-Meier method. The HRs comparing PAEPAD and standard care will be estimated using cox regression model. Multiple imputation will be conducted if loss to follow-up exceeds

30%. Subgroup analysis will be done for the relative risk of AD, asthma, rhinitis and food sensitization stratified by familial history of atopic disorders, and AD severity for allergic comorbidities (whenever applies). Regression models with interaction terms will be used to test for statistical significance among subgroups.

For sensitivity analysis that shall be done with the PP population, the analyses above will be conducted. For the sensitivity analysis that shall be done with the secondary analysis population, both traditional multivariate comparison and propensity score matching will be used to better balance the covariates and identify comparable groups. An additional sensitivity analysis will be conducted on the population that receives in-person education (as opposed to video recorded).

Data monitoring

An epidemiologist who is independent of the research team will be tasked to monitor the data. An interim analysis will be performed when 50% of the patients complete the one-year follow-up. The epidemiologist will conclude based on the interim analysis if the intervention is proved to be different from the control (standard management) and report to the Principal Investigator (PI). The PI can then decide whether or not to modify recruitment.

Patient and public involvement

No patient was involved with study design, recruitment or conduct.

Ethics and dissemination

The PAEPAD study is approved by the ethics committee of Capital Medical University Daxing Teaching Hospital. Written informed consent will be obtained from all participants. This study is registered at the Chinese Clinical Trial Registry under the identifier ChiCTR2000040463. Participation in the project is voluntary and will not impact the medical care of the women regardless of their participation status throughout pregnancy. All participants have the right to withdraw from the study at any point and have their data removed from the study database. All patient data will be securely stored and kept accessible by the research members only, with previous authorization from the PI. The results will be disseminated through peer-reviewed journals. Results will also be communicated at scientific conferences.

DISCUSSION

PAEPAD study is a large single-center randomized controlled trial of an antenatal educational intervention for prevention of atopic dermatitis and the atopic march. Previous studies on educational interventions have been therapeutic, aiming at reducing symptoms and improving quality of life. Therapeutic patient education (secondary prevention) in AD has been proved to be effective with a significant reduction in disease severity[21]. However, little is known about the primary preventive effect of educational and lifestyle interventions.

A strength of this study is the large sample size. In a similar study that aims to evaluate the effect of prenatal education on knowledge and behavioral changes for allergic disease prevention, the sample size was determined based on the behavioral matrices, resulting in a total sample size of 120 [36]. As behavioral changes do not necessarily modify disease outcomes, a larger sample size is warranted to provide adequate power to detect disease outcome differences. Another strength is that we chose the topics of the PAEPAD sessions based on both experts' opinion and a previous survey (Supplementary material, table S1), which indicated that new mothers needed help on infant skin care, safe practice of sun protection and building an unbiased understanding on common topical drugs, especially corticosteroids. We did not discuss about the treatment of atopic diseases further than emphasizing the importance of following the instructions of physicians on a guideline-oriented [2 13 31-34] management at this lecture. Recent pilot studies provided strong efficacy signals for the hypothesis that daily emollient use could prevent atopic dermatitis[15 17]. While the subsequent large trials yielded null results[12 14], it's crucial to realize that the hypotheses in these trials differ significantly. In the pilot studies, emollients were continued until the subsequent outcome assessment, whereas in the larger pragmatic trials, a washout period was implemented. In the Barrier Enhancement for Eczema Prevention (BEEP) study, the rates of continued emollient and wash product use extended beyond the intervention period until outcome assessment were as low as four and five percent for the intervention and the control group respectively[12]. Thus, the pilot studies assessed the immediate preventive effect, whereas the larger studies assessed whether this effect, if there is any, is sustainable. It's reasonable to hypothesize that the protective effect of emollients may not sustain beyond a year after refraining from application.

The current study will explore the preventive effect of an educational intervention, which bears a closer resemblance to the real world scenario through which behavioral changes are achieved. In addition, we plan to longitudinally collect biological specimens to study the crosstalk of lifestyle changes and molecular biology.

The study has some limitations. First, this study is subject to contamination due to the nature of a non-medication intervention and the single-center design. Consequently, the effect size to be detected will likely be a more conservative estimation of the real preventive effect. Therefore, we plan to collect cross-over data and conduct sensitivity analyses based on the PP population. Second, although the PAEPAD lectures will be led by experienced obstetricians and dermatologists, the heterogeneity may nonetheless constitute potential bias. We aim to reduce the heterogeneity by providing a training session and mock classrooms before the project launching during which lecturers will be evaluated on the organization of the class, clarity, student engagement and consistency of performance.

In conclusion, the PAEPAD study will add to our knowledge of the preventive effect of antenatal education on allergic disease outcomes and identify cellular and molecular changes that will warrant future studies. We expect that results from the PAEPAD study will expand our understanding of the primary prevention of allergic disorders.

AUTHORS' CONTRIBUTIONS

Lin Ma and Xiuhua Ma are the principal investigators of this trial and conceptualized the trial. Mutong Zhao, Yuan Liang, Jing Tian, Fengli Song, Ying Wang are responsible for the execution of the project. Mutong Zhao has written the first protocol manuscript. All authors critically reviewed the article. All the authors approved the final manuscript.

Authorship eligibility

Authorship of the consequent publication of this project will be granted to those who made a significant contribution to the conception, design, implementation, analysis of the data, or those who drafted the work or reviewed/revised it critically for important intellectual content.

Availability of data and materials

The datasets to be analyzed will be available from the corresponding author upon reasonable request (requests should be directed to muz880@mail.harvard.edu).

FUNDING

This study has received a public grant from the Capital's Funds for Health Improvement and Research (CFH2020-2-7121). The funders had no role in the design of this study and will not have any role during its execution, analyses, interpretation of data, or submission of outcomes.

COMPETING INTERESTS

None declared.

References

1. Muraro A, Halken S, Arshad SH, et al. EAACI food allergy and anaphylaxis guidelines. Primary prevention of food allergy. *Allergy* 2014;69(5):590-601. doi: 10.1111/all.12398
2. Wollenberg A, Barbarot S, Bieber T, et al. Consensus-based European guidelines for treatment of atopic eczema (atopic dermatitis) in adults and children: part I. *Journal of the European Academy of Dermatology and Venereology : JEADV* 2018;32(5):657-82. doi: 10.1111/jdv.14891
3. Bousquet J, Schunemann HJ, Fonseca J, et al. MACVIA-ARIA Sentinel NetworK for allergic rhinitis (MASK-rhinitis): the new generation guideline implementation. *Allergy* 2015;70(11):1372-92. doi: 10.1111/all.12686
4. Sasaki M, Koplin JJ, Dharmage SC, et al. Prevalence of clinic-defined food allergy in early adolescence: The SchoolNuts study. *The Journal of allergy and clinical immunology* 2018;141(1):391-98 e4. doi: 10.1016/j.jaci.2017.05.041
5. Paller AS, Spergel JM, Mina-Osorio P, et al. The atopic march and atopic multimorbidity: Many trajectories, many pathways. *The Journal of allergy and clinical immunology* 2019;143(1):46-55. doi: 10.1016/j.jaci.2018.11.006
6. Liang Y, Chang C, Lu Q. The Genetics and Epigenetics of Atopic Dermatitis-Filaggrin and Other Polymorphisms. *Clin Rev Allergy Immunol* 2016;51(3):315-28. doi: 10.1007/s12016-015-8508-5 [published Online First: 2015/09/20]
7. Chinthrajah RS, Hernandez JD, Boyd SD, et al. Molecular and cellular mechanisms of food allergy and food tolerance. *The Journal of allergy and clinical immunology* 2016;137(4):984-97. doi: 10.1016/j.jaci.2016.02.004 [published Online First: 2016/04/10]
8. Shimura S, Takai T, Iida H, et al. Epicutaneous Allergic Sensitization by Cooperation between Allergen Protease Activity and Mechanical Skin Barrier Damage in Mice. *The Journal of investigative dermatology* 2016;136(7):1408-17. doi: 10.1016/j.jid.2016.02.810 [published Online First: 2016/03/19]
9. Guttman-Yassky E, Zhou L, Krueger JG. The skin as an immune organ: Tolerance versus effector responses and applications to food allergy and hypersensitivity reactions. *The Journal of allergy and clinical immunology*

2019;144(2):362-74. doi: 10.1016/j.jaci.2019.03.021 [published Online First: 2019/04/08]
10. Chan SM, Turcanu V, Stephens AC, et al. Cutaneous lymphocyte antigen and alpha4beta7 T-lymphocyte responses are associated with peanut allergy and tolerance in children. *Allergy* 2012;67(3):336-42. doi: 10.1111/j.1398-9995.2011.02765.x [published Online First: 2012/03/17]
11. Deckers J, Bougarne N, Mylka V, et al. Co-Activation of Glucocorticoid Receptor and Peroxisome Proliferator-Activated Receptor-gamma in Murine Skin Prevents Worsening of Atopic March. *The Journal of investigative dermatology* 2018;138(6):1360-70. doi: 10.1016/j.jid.2017.12.023
12. Chalmers JR, Haines RH, Bradshaw LE, et al. Daily emollient during infancy for prevention of eczema: the BEEP randomised controlled trial. *Lancet* 2020;395(10228):962-72. doi: 10.1016/S0140-6736(19)32984-8 [published Online First: 2020/02/23]
13. Eichenfield LF, Tom WL, Berger TG, et al. Guidelines of care for the management of atopic dermatitis: section 2. Management and treatment of atopic dermatitis with topical therapies. *Journal of the American Academy of Dermatology* 2014;71(1):116-32. doi: 10.1016/j.jaad.2014.03.023 [published Online First: 2014/05/13]
14. Skjerven HO, Rehbinder EM, Vettukattil R, et al. Skin emollient and early complementary feeding to prevent infant atopic dermatitis (PreventADALL): a factorial, multicentre, cluster-randomised trial. *Lancet* 2020;395(10228):951-61. doi: 10.1016/S0140-6736(19)32983-6 [published Online First: 2020/02/23]
15. Horimukai K, Morita K, Narita M, et al. Application of moisturizer to neonates prevents development of atopic dermatitis. *The Journal of allergy and clinical immunology* 2014;134(4):824-30 e6. doi: 10.1016/j.jaci.2014.07.060
16. McClanahan D, Wong A, Kezic S, et al. A randomized controlled trial of an emollient with ceramide and filaggrin-associated amino acids for the primary prevention of atopic dermatitis in high-risk infants. *Journal of the European Academy of Dermatology and Venereology : JEADV* 2019;33(11):2087-94. doi: 10.1111/jdv.15786 [published Online First: 2019/07/10]
17. Simpson EL, Chalmers JR, Hanifin JM, et al. Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention. *The Journal of allergy and clinical immunology* 2014;134(4):818-23. doi: 10.1016/j.jaci.2014.08.005
18. Health behavior and health education: Theory, research, and practice, 4th ed. San Francisco, CA, US: Jossey-Bass 2008.
19. World Health Organization ROftEM. Health education: theoretical concepts, effective strategies and core competencies: a foundation document to guide capacity development of health educators, 2012.
20. Tan X, Feldman SR, Chang J, et al. Topical drug delivery systems in dermatology: a review of patient adherence issues. *Expert opinion on drug delivery* 2012;9(10):1263-71. doi: 10.1517/17425247.2012.711756
21. Zhao M, Liang Y, Shen C, et al. Patient Education Programs in Pediatric Atopic Dermatitis: A Systematic Review of Randomized Controlled Trials and Meta-

Analysis. *Dermatol Ther (Heidelb)* 2020;10(3):449-64. doi: 10.1007/s13555-020-00365-z [published Online First: 2020/03/23]
22. Sidbury R, Tom WL, Bergman JN, et al. Guidelines of care for the management of atopic dermatitis: Section 4. Prevention of disease flares and use of adjunctive therapies and approaches. *Journal of the American Academy of Dermatology* 2014;71(6):1218-33. doi: 10.1016/j.jaad.2014.08.038
23. Al-Ateeq MA, Al-Rusaiess AA. Health education during antenatal care: the need for more. *International journal of women's health* 2015;7:239-42. doi: 10.2147/IJWH.S75164
24. Parat S, Negre V, Baptiste A, et al. Prenatal education of overweight or obese pregnant women to prevent childhood overweight (the ETOIG study): an open-label, randomized controlled trial. *International journal of obesity* 2019;43(2):362-73. doi: 10.1038/s41366-018-0205-z
25. Suto M, Takehara K, Yamane Y, et al. Effects of prenatal childbirth education for partners of pregnant women on paternal postnatal mental health and couple relationship: A systematic review. *J Affect Disord* 2017;210:115-21. doi: 10.1016/j.jad.2016.12.025 [published Online First: 2016/12/27]
26. Nnam NM. Improving maternal nutrition for better pregnancy outcomes. *Proc Nutr Soc* 2015;74(4):454-9. doi: 10.1017/S0029665115002396 [published Online First: 2015/08/13]
27. Basra MK, Gada V, Ungaro S, et al. Infants' Dermatitis Quality of Life Index: a decade of experience of validation and clinical application. *The British journal of dermatology* 2013;169(4):760-8. doi: 10.1111/bjd.12563 [published Online First: 2013/08/06]
28. Eichenfield LF, Tom WL, Chamlin SL, et al. Guidelines of care for the management of atopic dermatitis: section 1. Diagnosis and assessment of atopic dermatitis. *Journal of the American Academy of Dermatology* 2014;70(2):338-51. doi: 10.1016/j.jaad.2013.10.010 [published Online First: 2013/12/03]
29. Mochizuki H, Kusuda S, Okada K, et al. Palivizumab Prophylaxis in Preterm Infants and Subsequent Recurrent Wheezing. Six-Year Follow-up Study. *Am J Respir Crit Care Med* 2017;196(1):29-38. doi: 10.1164/rccm.201609-1812OC [published Online First: 2017/02/06]
30. Ballardini N, Bergstrom A, Wahlgren CF, et al. IgE antibodies in relation to prevalence and multimorbidity of eczema, asthma, and rhinitis from birth to adolescence. *Allergy* 2016;71(3):342-9. doi: 10.1111/all.12798 [published Online First: 2015/10/28]
31. Wang H, Ma L, Tan Q, et al. Chinese Expert Consensus on the Diagnosis and Management of Food Allergy in Children With Atopic Dermatitis#. 2020;3(3):135-41. doi: 10.1097/jd9.0000000000000091
32. Okubo K, Kurono Y, Ichimura K, et al. Japanese guidelines for allergic rhinitis 2017. *Allergol Int* 2017;66(2):205-19. doi: 10.1016/j.alit.2016.11.001 [published Online First: 2017/02/19]
33. Scadding GK, Kariyawasam HH, Scadding G, et al. BSACI guideline for the diagnosis and management of allergic and non-allergic rhinitis (Revised Edition 2017; First edition 2007). *Clin Exp Allergy* 2017;47(7):856-89. doi: 10.1111/cea.12953 [published Online First: 2017/07/01]

34. Arakawa H, Hamasaki Y, Kohno Y, et al. Japanese guidelines for childhood asthma 2017. *Allergol Int* 2017;66(2):190-204. doi: 10.1016/j.alit.2016.11.003 [published Online First: 2017/01/22]
35. Guo Y, Li P, Tang J, et al. Prevalence of Atopic Dermatitis in Chinese Children aged 1-7 ys. *Scientific reports* 2016;6:29751. doi: 10.1038/srep29751
36. Nakamura R, Ishiguro N, Naru E, et al. The effect of an educational program for pregnant women to prevent allergic diseases in infants: study protocol for a randomized controlled trial. *Trials* 2019;20(1):755. doi: 10.1186/s13063-019-3797-2 [published Online First: 2019/12/23]

564

Table 1. Outcome assessments of the PAEPAD study

Outcomes	Instruments/Diagnostic criteria	Immediately post intervention	Upon discharge from OB ward	Upon disease flares	3 months postnatal	2 year postnatal
Primary outcome						
Cumulative incidence of AD at 2 years	Hanifin&Rajak					×
Secondary outcomes						
AD outcomes						
Time to first AD episode	Hanifin&Rajak			×		
Time to first topical corticosteroid exposure				×		
Disease related quality of life	IDQOL			×		
Disease severity	SCORAD, EASI and IGA			×		
Frequency of AD flares						
AD disease free days	IGA					
Cumulative clinical visit duration	From the parents being seated within the consulting room to their exit thereafter			×		
Atopic march outcomes						
Asthma incidence						×
Recurrent wheeze incidence						×

Rhinitis incidence						×
Food sensitization incidence	Allergen specific IgE					×
Obstetric outcomes						
Mode of birth	Eutocic/ dystocic/C-section		×			
Pathological pregnancy	Gestational diabetes, pre-eclampsia, placenta previa, placental abruption, prelabor rupture of membranes, puerperal infection, postpartum hemorrhage		×			
Neonatal outcomes:						
Newborn ,s weight			×			
Admission in neonatal care unit (yes/no).			×			
Apgar score			×			
Fetal growth retardation			×			
Any morbidity of the newborn that results in hospitalization in the first three month of life					×	
Patient disease knowledge and attitude changes	Questionnaire currently under validation	×		×	×	×

AD, atopic dermatitis; OB, obstetrics; IDQOL, Infantile Dermatitis Quality Of Life; SCORAD, SCORing of Atopic Dermatitis; EASI, Eczema Area and Severity Index; IGA, Investigator Global Assessment

565

Table 2. Biological sample collection at fixed follow-up visits

Samples	GW12	GW24-28	GW36	Delivery	24-72h post-partum	42 days post-partum	3 months post-partum	6 months post-partum	12 months post-partum	24 months post-partum
Biological sample of the infant										
Blood				×					×	×
Stool					×	×	×	×	×	×
Skin swab					×	×	×		×	×
Tongue dorsum swab				×	×	×	×	×	×	×
Tape stripping				×	×	×	×		×	×
Biological sample of the mother										
Blood		×	×	×	×					
Stool	×	×	×			×	×			
Urine	×	×	×		×	×	×		×	×
Skin swab		×	×		×	×	×			
Breast milk					×	×	×			
Placenta				×						
Tongue dorsum swab		×	×	×	×	×	×			

Vaginal swab	×	×		×

GW, gestational week

Figure legend

Fig.1 Flow diagram of the PAEPAD (Preventive Antenatal Educational Program on Allergic Diseases) study.

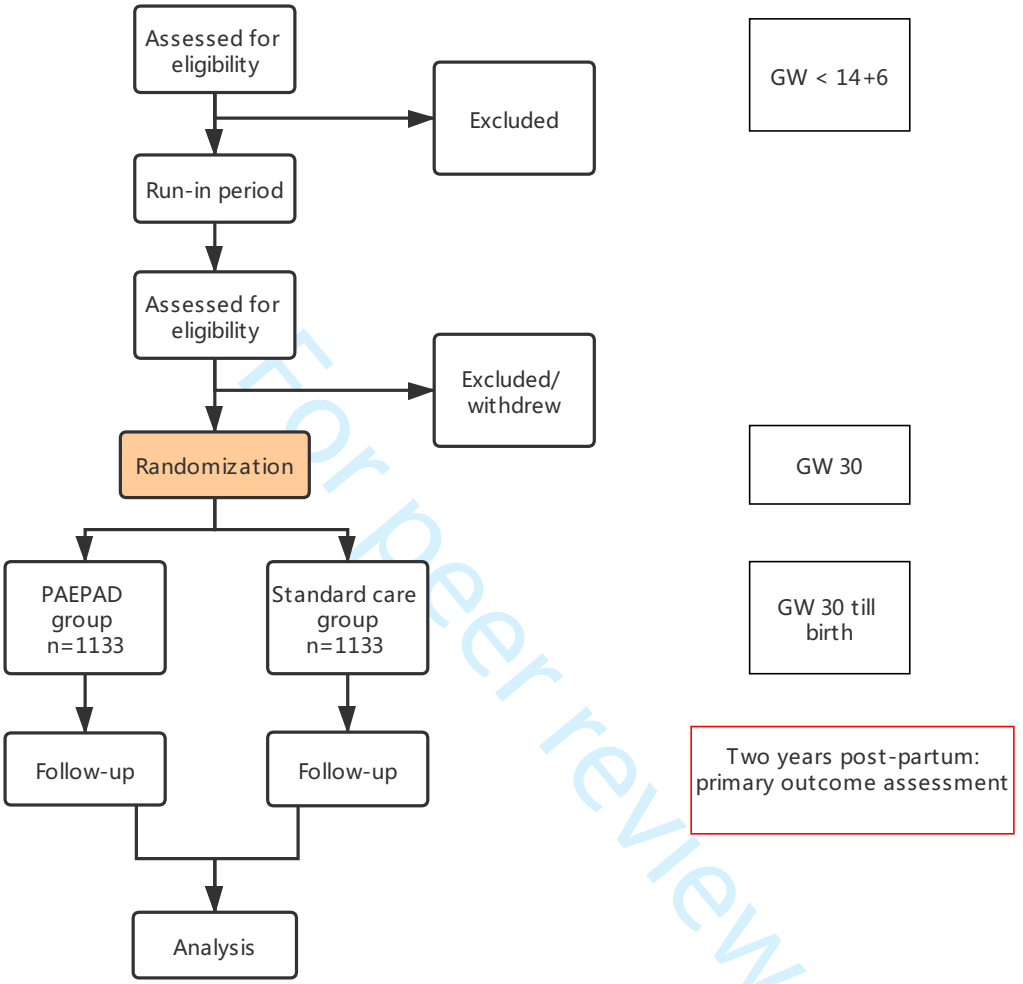

Table of Contents

BIOLOGICAL SAMPLE COLLECTION 2

TABLE S1. RESPONSES OF A 12-ITEM KNOWLEDGE SURVEY 6

BIOLOGICAL SAMPLE COLLECTION

Blood samples

Blood samples will be collected for analyses of DNA, protein, and exosomes and isolation of Peripheral Blood Mononuclear Cells (PBMCs). The mothers will be asked to be fasting for 8 h or more before the blood draw. Blood samples will be kept at room temperature for 30 min before centrifugation. For DNA analyses, a blood sample of 2ml will be collected at gestational week 24-28 into EDTA tubes and stored at -80°C until use. For protein analyses, the serum will be centrifuged at 1000g for 15 min at room temperature and the supernatants will be aliquoted and stored at − 80 °C until use. For PBMC isolation, PBMC will be purified as previously described, and stored in liquid nitrogen for use [1]. For exosome analyses, 5ml of blood will be collected into EDTA tubes and stored at 4°C for 4 h or overnight. Plasma will be separated by centrifugation at 5000×g for 5 minutes at 4°C. Cell-free, platelet poor plasma will be collected, aliquoted and stored at − 80 °C until use[2].

At birth, the venous and arterial cord blood will be collected from the umbilical cord into EDTA tubes using a syringe. Blood components will be processed and stored as detailed above for analyses of DNA, protein and exosomes. An additional 3mL arterial cord blood will be drawn and stored at room temperature for flow cytometric analysis.

At the year of two, children will be asked to test for serum specific allergens including house dust mite, cat dander, birch pollen, grass pollen, milk, and egg. If the parent consent to having their children's blood drawn, 2ml blood will be collected into tubes containing clot activator and centrifuged at 1000g for 15 min. The supernatant will be aliquoted into 500ul aliquotes and stored at − 80 °C until use.

Urine samples

The first catch midstream urine of mothers will be collected using a sterile cup, centrifuged and added to sterile tubes and stored at –80 °C until analyzed.

Placenta

After delivery, the placenta will be sampled for analyses of histology, DNA, methylation, RNA and protein. First, a cross-section 2cm away from the cord insertion will be sampled and stored in formalin to be fixed for histological examination. Next, twenty pieces of villous tissue on the maternal aspect measuring 3*3*3mm will be taken at 2-4cm away from cord insertion and stored in five cryo-tubes, among which two will be prefilled with RNAlater. Villous samples will be snap-frozen in liquid nitrogen and then stored at -80°C. Third, villous tissue on the fetal aspect will be sampled and stored likewise.

Breast milk

Breast milk will be collected with a breast pump at locations where postnatal follow-ups take place. At follow-up, 30ml of breast milk will be collected with sterilized RNase-free tubes, with the first 500ul disposed. For exosome analysis, 15 ml breast milk will be centrifuged at 4°C for 10 minutes at 1500 g to remove cells and the cream layer. The supernatant will then be transferred to new tubes and centrifuged again at 12,000 g at 4°C to remove remaining cells and cream. The supernatant will then be immediately processed or frozen at -80 °C until use[3]. The remaining 15 ml breast milk sample will be aliquoted and stored at -80 °C directly until use.

Feces

The fecal samples will be self-collected by participants. For 16s RNA sequencing, participants will be provided with a sterile feces collection and preservation kit (ML-001A, Shenzhen Dayun Gene Technology Co., Shenzhen, China). For metabolomics analyses, participants will be provided with a sterile feces collection device with a spatula. Participants will be instructed to collect stool specimens of approximately 10ml within 2 h before each visit and bring them to the visits. Collected feces will then be snap-frozen in liquid nitrogen and stored at -80 °C until use [4]. Meconium of 2ml will be sampled and stored likewise.

Vagina swab

Vaginal swabs (4520CA, COPAN Flock Technologies, Brescia, Italy) will be collected from the posterior fornix by obstetricians. A speculum will be placed in the absence of lubrication. All specimens will be collected by swirling a sterile swab for 30 seconds, withdrawing the swab without contamination from other sites, and transferring the specimen into a sterile tube and stored at -80 °C until use.

Skin swab

Skin specimens will be collected from the cheek, anterior forearm, and lesional sites of the children. The forehead, anterior forearm, and cubital fossa of the mother will be sampled likewise. These sites will be swiped vigorously for 50 times over an area of 4 cm2 using a sterile swab (4520CA, COPAN Flock Technologies, Brescia, Italy). The swab will be premoistened with DNA free saline. Swab specimens will be inserted into a sterile tube and stored at -80 °C until use.

Tape stripping

For each child, skin lipids will be collected by tape stripping from the same body sites as to where skin swabs are sampled from the children. The first layer will be discarded, and the second to fourth layers at the same place will be retained and placed separately in a glass tube

with 5 mL of methanol, then stored at -80 °C until use. The vernix will be sampled from the back of the newborn likewise.

Tongue dorsum swab

Subjects will be instructed not to eat, drink (except water), or brush their tongue during the 12 h period before sampling and not to brush their teeth during the 2 h period before sampling. Specimens will be collected from the central part of the tongue dorsum by swiping for 15 seconds using a sterile swab (4520CA, COPAN Flock Technologies, Brescia, Italy).

References

1. Riedhammer C, Halbritter D, Weissert R. Peripheral Blood Mononuclear Cells: Isolation, Freezing, Thawing, and Culture. *Methods Mol Biol* 2016;1304:53-61. doi: 10.1007/7651_2014_99 [published Online First: 2014/08/06]
2. Stranska R, Gysbrechts L, Wouters J, et al. Comparison of membrane affinity-based method with size-exclusion chromatography for isolation of exosome-like vesicles from human plasma. *J Transl Med* 2018;16(1):1. doi: 10.1186/s12967-017-1374-6 [published Online First: 2018/01/11]
3. Zonneveld MI, Brisson AR, van Herwijnen MJ, et al. Recovery of extracellular vesicles from human breast milk is influenced by sample collection and vesicle isolation procedures. *J Extracell Vesicles* 2014;3 doi: 10.3402/jev.v3.24215 [published Online First: 2014/09/11]
4. Costea PI, Zeller G, Sunagawa S, et al. Towards standards for human fecal sample processing in metagenomic studies. *Nature biotechnology* 2017;35(11):1069-76. doi: 10.1038/nbt.3960

Table S1. Responses of a 12-item Knowledge survey

Survey questions	Score	Number answered (n=395), n (%)
1. I should apply body wash every time I bathe my baby.	1	17 (4.30)
	2	61 (15.44)
	3	61 (15.44)
	4	124 (31.39)
	5	132 (33.42)
2. Moisturizer should be applied all over my baby's body.	1	27(6.84)
	2	86(21.77)
	3	76(19.24)
	4	128(32.41)
	5	78(19.75)
3. Moisturizer should be used no more than once a day.	1	55 (13.92)
	2	109 (27.59)
	3	121 (30.63)
	4	91 (23.04)
	5	19 (4.81)
4. I only use moisturizer after I bathe my baby.	1	25(6.33)
	2	65(16.46)
	3	79(20.00)
	4	170(43.04)
	5	56(14.18)
5. Massage oils can be used as a baby moisturizer.	1	32 (8.10)
	2	96 (24.30)
	3	112 (28.35)
	4	112 (28.35)
	5	43 (10.89)
6. Moisturizer is not necessary for my baby at summer time.	1	10(2.53)
	2	36(9.11)
	3	72(18.23)
	4	160(40.51)
	5	117(29.62)
7. Sweat is an irritant to the baby's skin.	1	39 (9.87)
	2	54 (13.67)
	3	95 (24.5)

	4	105 (26.58)
	5	102 (25.82)
8. I'm concerned to use topical corticosteroids ointment on my baby.	1	103(26.08)
	2	81(20.51)
	3	149(37.72)
	4	37(9.37)
	5	25(6.33)
9. Topical corticosteroids will make the baby fat.	1	43(10.89)
	2	53(13.42)
	3	124(31.39)
	4	105(26.58)
	5	70(17.72)
10. Topical corticosteroids will induce premature puberty in babies.	1	69(17.47)
	2	47(11.9)
	3	138(34.94)
	4	88(22.28)
	5	53(13.42)
11. If I had to use topical corticosteroids on my baby, I'd be concerned that my baby will become addicted to the drug.	1	93(23.54)
	2	67(16.96)
	3	140(35.44)
	4	59(14.94)
	5	36(9.11)
12. If I had to use topical corticosteroids on my baby, I'd be concerned that my baby will become resistant to the drug.	1	88(22.28)
	2	89(22.53)
	3	135(34.18)
	4	57(14.43)
	5	26(6.58)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Page No.
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3, Line 51
	2b	All items from the World Health Organization Trial Registration Data Set	Refer to the appendix
Protocol version	3	Date and version identifier	6, Line 144
Funding	4	Sources and types of financial, material, and other support	15, Line 418
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	14 Line 404
	5b	Name and contact information for the trial sponsor	15, Line 418
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	15 Line 420
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12 Line 334
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3 Line 61
	6b	Explanation for choice of comparators	5 Line 125
Objectives	7	Specific objectives or hypotheses	5 Line 126
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5 Line 125
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5 Line 136
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6 Line 169
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8 Line 201
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	Non pharmacological intervention. NA.
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	8 Line 201- 6
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9 Line 260

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8 Line 228
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Fig.1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11 Line 294
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6 Line 148
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	7 Line 187
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	7 Line 193
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7 Line 187

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7 Line 196
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Non pharmacological intervention. NA.
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	8 Line 227
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10 Line 269
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10 Line 269
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14 Line 392
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14 Line 406
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13 Line 381
Methods: Monitoring			

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12 Line 334
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12 Line 337
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Non pharmacological intervention. NA.
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Not reported
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	12 Line 344
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6 Line 153
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	6 Line 153
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	10 Line 283

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15 Line 422
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10 Line 283, 12 Line 350
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	None
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12 Line 343
	31b	Authorship eligibility guidelines and any intended use of professional writers	14 Line 409
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not reported
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Available upon request
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	10 Line 287, supplementary material

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

Appendix. World Health Organization Trial Registration Data Set

Data category	Information
Primary registry and trial identifying number	ChiCTR registry (Trial ID: ChiCTR2000040463)
Date of registration in primary registry	28 November, 2020
Secondary identifying numbers	CFH2020-2-7121
Source(s) of monetary or material support	Capital's Funds for Health Improvement and Research
Primary sponsor	Capital's Funds for Health Improvement and Research
Secondary sponsor(s)	NA
Contact for public queries	Mutong Zhao, MD, MSc. [+86 18600116126r] [muz880@mail.harvard.edu]
Contact for scientific queries	Lin Ma, MD, Department of Dermatology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China. E-mail: bch_maleen@aliyun.com. Tel: +86 13601305676 Xiuhua Ma, MD, Department of Obstetrics and Gynecology, Beijing Daxing District People's Hospital, Capital Medical University Daxing Teaching Hospital, Beijing, China. E-mail: mxhdxqyy@126.com Tel: +86 13381021859
Public title	Preventive Antenatal Educational Program on Allergic Diseases (PAEPAD) for prevention of atopic dermatitis
Scientific title	Preventive Antenatal Educational Program on Allergic Diseases (PAEPAD) versus standard antenatal care for prevention of atopic dermatitis: study protocol for a single-center, investigator blinded randomized controlled trial.
Countries of recruitment	China
Health condition(s) or problem(s) studied	Atopic dermatitis
Intervention(s)	PAEPAD group: *multi-disciplinary education of neonatal care* Standard care group: standard education of neonatal care
Key inclusion and exclusion criteria	Ages eligible for study: ≥18 years Sexes eligible for study: expecting mothers Accepts healthy volunteers: no Inclusion criteria: 1. Enlisted in the birth registry of Daxing Teaching Hospital of Capital Medical University and intend to give birth at this location; 2. Women aged ≥18; 3. Less than 14+6 gestational week when recruited as measured by last menstrual period; 4. Residents of

	Daxing and intend to remain residing in Daxing for at least two years postpartum; 5. Written consent form. Exclusion criteria: 1. Planned abortion; 2. Rare comorbidities of the mother that may cause miscarriage and congenital disabilities as determined by obstetricians, including but not limited to malignancies, congenital heart diseases, monogenetic diseases; 3. Recurrent miscarriage.
Study type	Interventional Allocation: randomized Intervention model: parallel assignment Masking: investigator blind Primary purpose: prevention
Date of first enrolment	28 November, 2020
Target sample size	2266
Recruitment status	Recruiting
Primary outcome(s)	Cumulative incidence of atopic dermatitis at 2 years
Key secondary outcomes	Atopic dermatitis outcomes, atopic march outcomes, obstetric outcoms, neonatal outcomes, parental knowledge outcomes as detailed in the manuscrips
Ethics review	Approved on 23 November, 2020 by the ethical committee of Capital Medical University Daxing Teaching Hospital, Beijing, China.
Completion date	Last subject, last visit
Summary results	NA
IPD sharing statement	IPD will be shared with upon request (requests should be directed to muz880@mail.harvard.edu) for individual data meta-analyses (time frame: 1 year after publication. No end date.).

BMJ Open

Preventive Antenatal Educational Program on Allergic Diseases (PAEPAD) versus standard antenatal care for prevention of atopic dermatitis: study protocol for a single-center, investigator blinded randomized controlled trial.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-048083.R1
Article Type:	Protocol
Date Submitted by the Author:	28-Aug-2021
Complete List of Authors:	zhao, mutong; Beijing Children's Hospital Capital Medical University, Dermatology Liang, Yuan; Beijing Children's Hospital Capital Medical University, Dermatology Song, Fengli; Capital Medical University Daxing Teaching Hospital Ma, Lili; Capital Medical University Daxing Teaching Hospital Wang, Ying; Capital Medical University Daxing Teaching Hospital Gao, Wanli; Capital Medical University Daxing Teaching Hospital Tian, Jing; Beijing Children's Hospital Capital Medical University, Dermatology Ying, Xiangji; Peking University Cancer Hospital Shen, Chunping; Beijing Children's Hospital Capital Medical University Wang, Shan; Beijing Children's Hospital Capital Medical University Jiao, Lei; Beijing Children's Hospital Capital Medical University Wang, Yang; Beijing Children's Hospital Capital Medical University Sun, Xiaoyan; Capital Medical University Daxing Teaching Hospital Ma, Lin; Beijing Children's Hospital Capital Medical University, Dermatology Ma, Xiuhua; Capital Medical University Daxing Teaching Hospital
Primary Subject Heading:	Dermatology
Secondary Subject Heading:	Dermatology
Keywords:	Eczema < DERMATOLOGY, EPIDEMIOLOGY, PAEDIATRICS, Paediatric dermatology < PAEDIATRICS

TITLE PAGE

Preventive Antenatal Educational Program on Allergic Diseases (PAEPAD) versus standard antenatal care for prevention of atopic dermatitis: study protocol for a single-center, investigator-blinded randomized controlled trial.

Mutong Zhao[1], Yuan Liang[1], Fengli Song[2], Lili Ma[2], Ying Wang[2], Wanli Gao[2], Jing Tian[1], Xiangji Ying[3], Chunping Shen[1], Shan Wang[1], Lei Jiao[1], Yang Wang[1], Xiaoyan Sun[4], Lin Ma[1], Xiuhua Ma[2]

1 Department of Dermatology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China, 100045.

2 Department of Obstetrics and Gynecology, Beijing Daxing District People's Hospital-Capital Medical University Daxing Teaching Hospital, Beijing, China, 102600.

3 Gastrointestinal Cancer Center, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Peking University Cancer Hospital and Institute, Hai-Dian District, Beijing, China, 100142

4 Department of Dermatology and Venerology, Beijing Daxing District People's Hospital-Capital Medical University Daxing Teaching Hospital, Beijing, China, 102600.

Correspondence to:

Lin Ma, MD, Department of Dermatology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China. E-mail: bch_maleen@aliyun.com. Tel: +86 13601305676

Xiuhua Ma, MD, Department of Obstetrics and Gynecology, Beijing Daxing District People's Hospital, Capital Medical University Daxing Teaching Hospital, Beijing, China. E-mail: mxhdxqyy@126.com Tel: +86 13381021859

Word count: 4407

ABSTRACT

Introduction

Patient education serves an essential purpose in the long-term management of allergic diseases as a secondary prevention approach. However, evidence on using education for primary prevention is limited. This study aims to evaluate the effect of an educational intervention, i.e., the Preventive Antenatal Education Program on Allergic Diseases (PAEPAD), on infantile allergic disease incidences compared with the standard care.

Methods and analysis

This is a single-center randomized controlled trial of expecting mother-children dyads in Daxing Teaching hospital of Beijing, China. A total of 2266 expecting mothers will be recruited. Expecting mothers enlisted in the birth registry of Daxing Teaching Hospital of Capital Medical University and intend to give birth at this location will be screened for eligibility. Women aged ≥18 with less than 14+6 weeks of pregnancy who intends to remain resident in Daxing district for at least two years postpartum will be entered into the run-in phase. Randomization will take place at 30 weeks of gestation. Women at high risk for miscarriage or intend to have abortions will be excluded. The participants will be allocated into two groups (i.e., the PAEPAD and the standard care group) by random allocation (1:1). The PAEPAD group will receive a multi-disciplinary education of neonatal care, including standard education as the control group and additional information on skincare of infants, sun protection, topical corticosteroids, and an overview of atopic dermatitis; whereas the standard care group will receive the standard neonatal care education carried out by obstetricians. Participants will be followed for two years. The primary outcome will be infantile atopic dermatitis (AD) cumulative incidence at two years post-partum. Secondary outcomes will include other AD outcomes, atopic march outcomes, knowledge outcomes, and other maternal and neonatal outcomes. Data collection will be carried out using both electronic and paper questionnaires. Biological samples will also be collected longitudinally.

Ethics and dissemination

The study design was approved by the ethical committee of Capital Medical University Daxing Teaching Hospital, Beijing, China. The trial results will be published in peer-reviewed journals and at conferences.

Trial registration

The trial is prospectively registered at the ChiCTR registry (Trial ID: ChiCTR2000040463).

Strengths and limitations of the study

- Large single-center investigator-blinded randomized controlled trial (RCT).
- First attempt to investigate education for primary prevention of incident cases of allergic diseases (atopic dermatitis for primary outcome).
- Prone to potential contamination due to single-center design and non-medication intervention.
- Potential heterogeneity of intervention effect due to variation in how the intervention will be carried out by individual educator albeit provision of formal training for educators.

KEYWORDS

Primary prevention, atopic dermatitis, atopic march, health education, antenatal education, pediatric, study protocol

INTRODUCTION

Atopic disorders place a substantial burden on both individuals and the health care system[1-4]. The sequential occurrence of AD, followed by one or more disorders characterized by allergen-specific type 2 (including TH2) responses are designated as atopic march[5]. The underlying mechanisms feature both a genetic susceptibility in which barrier dysfunction and immune dysregulation predispose atopic individuals to Th2 immunity, and a progression in which AD and its pathological changes function as instigating events to subsequent development of other atopic comorbidities[5 6]. The human skin is a functional immune organ with an abundance of immunocompetent cells. In the resting state, an intact skin barrier and a balance of immune cell populations, cytokines, and chemokines promote immune tolerance, which is otherwise disrupted in AD patients. Barrier dysfunction from both loss-of-function mutations in filaggrin and the itch-scratch cycle was postulated as the driving component for atopic march programming by promoting Th2 and Th17 differentiation[7-9]. Subsequently, sensitizations to food allergens develop in the setting of Th2 skewing, which is otherwise Th1 skewed in the gastrointestinal-homing T cells in tolerant subjects[10]. Similarly, in asthmatic airways,

disrupted junctional adhesion, mucus plugging develops due to Th2 polarization. While the severity of subsequent allergic airway inflammation was affected by AD, abrogation of AD by topical treatment prevents worsening of subsequent airway inflammation by counteracting the Th17 pathway [11]. Granted that both the genetic predisposition and the atopic progression contribute to the development of atopic march, longitudinal studies are warranted to explore the impact of immune activation of AD on the development of other TH2 comorbidities. Meanwhile, the role of epithelial-immune crosstalk in the atopic march needs to be further elucidated.

To date, extensive research has been conducted on the therapeutic effect of barrier enhancement using emollients. Compelling evidence indicated that emollients render the skin less susceptible to irritants and reduce flares of AD (secondary prevention)[12] and therefore was regarded as a cornerstone of AD therapy[13]. However, evidence was inconsistent regarding the preventive effect of emollient application [12 14-17]. The discordance from these behavioral intervention studies is probably a result of the difference in when the outcome assessment took place. In the BEEP[12] and PreventADALL[14] studies, the outcome was measured after a long washout period to ensure that any mild AD would not be concealed by the on-going application of emollients, whereas in the small studies [15 16] the immediate effect was evaluated. However, the null long-term result should not overshadow the strong efficacy signal displayed at the immediate outcome assessments. Moreover, as behavior change is a process that unfolds through a continuum of stages from pre-contemplation, contemplation, preparation, to action and maintenance, such straightforward behavioral intervention may not echo with the real-world scenario (the stages of change model), where behavior changes are achieved through health promotion initiatives, including health education and health policies [18 19]. Consequently, the generalizability of these studies might be affected due to the difference in how intervention was delivered and the setting under which outcome assessment took place.

In realizing the tremendous burden inflicted by atopic disorders and the critical role of AD in atopic march, significant therapeutic discoveries have been made over the past century. Nonetheless, in AD and other chronic diseases alike, adherence to treatment can be strikingly poor [20]. The underlying factors were postulated to be the complexity of

treatment regimen, corticosteroid phobia, and caregiver burden [20]. Addressing these issues calls for consistent efforts in patient education that can hardly be accomplished in a typical clinical visit. Recent studies have shown that structured patient education can significantly reduce disease severity in AD patients [21]. Given this, guidelines for AD have acknowledged education of patients and caregivers as an essential form of secondary prevention to reduce disease flares and improve quality of lives[22]. However, little is known about how education performs as a primary prevention approach to reduce AD disease burden.

Antenatal education is a crucial component of antenatal care. Since its implementation from the nineteenth century, mortality in children has decreased tremendously. In 2005, the World Health Organization called for "realizing the Potential of Antenatal Care"[23], and thence started using it as a platform for primary prevention of malnutrition, HIV/AIDS, sexually transmitted infections, tuberculosis, and prevention of postpartum and neonatal diseases [24-26]. Its diversified usage has led us to hypothesize that educational intervention delivered through the antenatal education platform may yield informative data for assessing the primary prevention of allergic diseases. Thus, the Preventive Antenatal Education Program on Allergic Diseases (PAEPAD) study focuses on using the antenatal care platform as a more affordable and effective health education approach. In this investigator-blinded, randomized controlled trial, we aim to evaluate the effect of such intervention vs. standard education on atopic disease outcomes. The primary objective is to evaluate the preventive effect of PAEPAD on atopic dermatitis and atopic comorbidity incidences. The secondary objectives are: 1) to explore the immune-barrier crosstalk between the immune system, commensal flora and skin barrier function that may explain the development of atopic march; 2) to identify biomarkers (metabolites, MicroRNAs) that can be used to characterize individuals at high risk of AD and atopic march; 3) to elucidate whether and to what extent the maternal immune milieu influences pediatric AD development through cord blood and breast milk.

METHODS AND ANALYSIS

Study design

The study is a prospective investigator-blinded randomized controlled trial with two arms (PAEPAD vs. standard antenatal education). All expectant mothers planning to give birth

at the Daxing Teaching Hospital of Capital Medical University will be invited to participate. Daxing Teaching Hospital of Capital Medical University is a general hospital located in Daxing district of Beijing, China, with more than 6,000 deliveries annually. Daxing district makes up approximately 6.3% of Beijing geographically. Daxing Teaching Hospital of Capital Medical University makes up approximately 2.7% of Beijing's annual deliveries. This protocol was drafted (Aug 17th, 2020) before participant recruitment (Oct 30th, 2020).

RECRUITMENT, INCLUSION AND EXCLUSION CRITERIA

All expectant parents living in the catchment area of the Daxing Teaching Hospital of Capital Medical University will receive an information leaflet about the study at their first visit to the maternity clinic. Recruitment will take place in their first mandatory maternity class between gestation week 7 to 14+6. All expectant mothers will be given detailed information about the study. A trained research nurse will outline key information (i.e., inclusion and exclusion criteria, scheduled research visits, and instructions on biological sample collection) about the study in the form of a short lecture session. The parents who wish to participate will be requested to provide written informed consent. They will be inquired on five levels of consent: 1) consent to participate in the study; 2) consent to biological sample collections of the mother that are non-invasive, including but not limited to the recollection of blood from routine pregnancy workups; 3) consent to biological sample collections of the mother that are minimally invasive or could potentially cause discomfort, i.e., additional blood draw and vaginal swabs; 4) consent to biological sample collections of the child that are non-invasive, including but not limited to the recollection of blood from routine checkups; 5) consent to biological sample collections of the child that are minimally invasive or could potentially cause discomfort, i.e., additional blood draw and tape stripping. At birth, another consent form on the children's biological sample collection will be signed to allow for any change of consent status on non-invasive and minimally invasive sample collections. Consented participants will subsequently receive a QR code through their cellphone after registration. By scanning the QR code, the recruitment staff will be able to confirm their participation status, at the end of which a study-specific ID number will be generated automatically.

Inclusion and exclusion criteria

Run-in phase inclusion criteria: 1. Enlisted in the birth registry of Daxing Teaching Hospital of Capital Medical University and intend to give birth at this location; 2. Women aged ≥18; 3. Less than 14+6 gestational week when recruited as measured by last menstrual period; 4. Residents of Daxing and intend to remain residing in Daxing for at least two years postpartum; 5. Written consent form.

Randomization phase inclusion criteria:

Pregnant women enlisted in the birth registry of Daxing Teaching Hospital of Capital Medical University and intend to give birth at this location; passes the run-in phase criteria.

Withdrawal criteria: 1. Still birth; 2. Abortion (spontaneous or induced); 3. Rare comorbidities that present after inclusion into the study that may render the participant unsuitable for participation, including but not limited to malignancies, amniotic fluid embolism, eclampsia, and major birth abnormalities of the child.

Exclusion criteria: 1. Planned abortion; 2. Rare comorbidities of the mother that may cause miscarriage and congenital disabilities as determined by OBs, including but not limited to malignancies, congenital heart diseases, monogenetic diseases; 3. Recurrent miscarriage; 4. Mental, psychological or intellectual disabilities of either one of the expecting parents.

Treatment allocation

The study flow is as follows (Fig. 1): Participants will be randomly assigned to one of the two arms (i.e., PAEPAD vs. standard antenatal care). Randomization will be conducted by an epidemiologist using a computer-generated list with the number of groups being 2 and the distribution ratio of the two groups being 1:1. The list will be generated using block randomization with block sizes hidden from all investigators. Group allocation will be placed in sealed opaque envelopes, labeled by numbers only. The envelopes will be opened in consecutive order. Participants will then be informed about their allocated groups by a research nurse.

Blinding

This is a researcher-blinded study. Treatment allocation will be performed by an epidemiologist, and the researchers who evaluate the outcome matrices and analyze data

will be blinded and work independently from the group of clinicians who will carry out the intervention. Data entry will be undertaken by trial administrators blinded to group allocation.

Intervention

After randomization at GW 30, the participants will be informed on their allocation, and members of the research team will send out weekly invitations through messages to participants who have not yet completed the intervention. For those who failed to complete the intervention prior to admission into the OB department, a pre-recorded video will be played during their hospital stay. In the standard care group, patients will receive the standard neonatal care session from an experienced obstetrician, which will include breastfeeding, newborn screening tests, infant physiology, immunization, solid food introduction, belly and eye care (45 min). This session is one of the five mandatory sessions with participation of 83.7-91.9% over the past five years (unpublished data). The treatment group will receive an educational program designed by a multi-disciplinary group of experienced obstetricians and pediatric dermatologists. The program will be focused on neonatal care as the control group (45 min) and 1) Skin care of the newborns with a practical demonstration on bathing and emollient application (20 min); 2) Sun protection (3 min); 3) A brief introduction on commonly used topical agents during infancy, including topical corticosteroids, antibiotics, and astringents (5 min); 4) Besides, the program will also contain a 5 minutes presentation on atopic dermatitis disease burden, its precipitators, managements, disease courses, and the atopic march. Specific recommendations are listed in the supplementary table S1. At the neonatal care class, all participants will first receive the standard education, which will be held nonconcurrently to minimize group contamination. Online courses will be held whenever gathering are restricted due to the COVID-19 pandemic. At the end of the sessions, participants of the PAEPAD cohort will be asked to participate in the PAEPAD session, which will last for less than 40 min. Participants of the standard neonatal care cohort will be asked to leave. The intervention will be entirely educational, no cleanser or emollient product will be provided or recommended. At follow-up visits, no further education will be implemented. Any crossover and non-compliance will be surveyed by a research nurse at the beginning

and end of the antenatal sessions. The research nurses who collect data on compliance and who send out invitations will not be involved in outcome assessment.

Study outcomes and follow-up

Study outcomes

All outcome measures are summarized in Table 1. There will be both fixed and disease prompted postnatal follow-up visits during which outcome assessments will take place. Diagnostic criteria for AD will be based on Hanifin&Rajka criteria, which is regarded as the 'gold standard' for hospital-based research. The Infants' Dermatitis Quality of Life Index (IDQoL) is a questionnaire of ten items that has been translated into 21 languages. This questionnaire is validated in infants aged 0–3 years[27]. Disease severity at disease flares will be measured by SCORing of Atopic Dermatitis (SCORAD) and Eczema Area and Severity Index (EASI) and through the course of the disease by Investigator's Global Assessment (IGA). SCORAD and EASI are validated instruments for disease severity assessment [28]. IGA provides the most straightforward assessment of disease severity and will thus be employed to assess AD disease-free days. An IGA of less than two is defined as clearance of disease. An episode of expiratory wheezing will be defined as bronchial obstruction lasting for at least 24 hours preceded by at least a one-week non-wheezing healthy period, as defined by a physician. Recurrent wheezing will be defined as the occurrence of 3 or more episodes of expiratory wheezing diagnosed by a physician in a 12-month period[29]. Rhinitis will be defined as symptoms of sneezing, a runny or blocked nose, or itchy, red and watery eyes after exposure to furred pets or pollen the year before follow-up and/or doctor's diagnosis of allergic rhinitis[30]. Sensitization will be defined as allergen-specific IgE $\geq$0.35 kU_A/l. Parental knowledge and attitude will be quantified using a questionnaire. The items of the questionnaire were developed following rigorous procedures, including a review of literature, a patient focus group and a panel discussion of experts. This questionnaire is currently under validation (unpublished data).

Follow-up

Participants will receive both fixed and disease prompted follow-up visits. Follow-up visits will be carried out at Daxing hospital and five health service centers during immunization. At follow-ups, biological samples, surveys and physical examination data will be collected. Specific items are listed in Table. 2. In addition, children will be

assessed upon disease onsets and flares. During follow-ups, all cases will be treated according to the guidelines [2 13 31-34] by specialists who are actively engaged in the care of pediatric AD patients. Participants are not allowed to participate in other clinical trials after inclusion into the study till the end of the last follow-up visit

Covariates

Relevant covariates will include age, sex, social-economic status, familial history of allergic diseases, administration of systemic medication and nutrient supplements, maternal psychological status measured with Kessler-10 prenatally. Other variables, including the Edinburgh postnatal depression scale (EPDS), maternal comorbidities during pregnancy, postnatal nutrition status and indicators for feeding practices (i.e., minimum dietary diversity, the introduction of solid, semi-solid or soft foods, duration of breastfeeding) will also be collected.

Data collection and management

Data collection at fixed time points will be conducted with an electronic database designed specifically for this project. At each visit, patients will be asked to present a patient-specific QR code, by scanning which two different questionnaires will be delivered to the participants and a research nurse separately. Less than 15 items will be surveyed in a standard questionnaire for the patient to minimize respondent fatigue caused by lengthy questionnaires. The rest of the relevant data will be collected by the research nurses during a face-to-face interview. Routine lab workups will be collected from the participants' medical records. Data will be checked by the members of the research team, and incorrect or missing questions will be sent back to the participants. All data recorded in this electronic database will be accessible only by the team members. Participants will receive text reminders prior to each follow-up visit and will be interviewed through phone calls for incomplete visits. Data collection at disease onsets and flares will be carried out with paper surveys, which will be encrypted and kept accessible only by team members with authorization. Group allocation data are accessible through unique identifiers on a separate sheet by only the principal investigators. All data handling (data entry, storage, and analysis) will be confidential. The principal investigators are responsible for ensuring data quality.

Biological sample collection

At each time point, biological samples from mothers and children (where applies) will be collected and stored (Table 2). These samples include maternal blood, urine, feces, skin, vaginal and oral swabs, breast milk, placenta, cord blood, meconium, and blood, feces, skin, nasopharyngeal and external auditory canal swabs, tape stripping of skin lipids from the children. Procedures are detailed in the supplementary material.

Sample size

The PAEPAD study will be based on a sample of 2266 expecting mothers. We calculated that assuming 20% [35] AD rate, 20% lost to follow up (LTFU), with a clinically significant estimate of a cumulative incidence ratio of 0.75, 80% power and two-sided 5% significance level, the estimated sample size of the primary outcome would be 2266. The cutoff value of clinical significance of such educational intervention was derived by a survey of expert opinion (n=7, unpublished data) and from previous RCTs with behavioral interventions [15 17].

Analyses

Definition of population sets

1) Primary analysis population: The Modified Intent-to-treat Population (MITTP), which will comprise of expectant women who undergo randomization, with data of at least one post-intervention follow-up.
2) Per-protocol Population (PPP): All expectant women complying with the study protocol, with data of at least one post-intervention follow-up.
3) As treated population: All randomized participants who received the intervention (whether complied or not) , with data of at least one post-intervention follow-up.

Statistical analysis

Statistical analyses will be performed using STATA 14.0, R 1.0.44 and SAS9.2 statistical software.

The primary analysis will be based on the MITT population. Sensitivity analyses will be done with both the PP population and the as-treated population. For primary analyses, we will use χ^2 tests to compare categorical outcomes. For continuous variables, normally distributed continuous variables will be compared using the t-test, and the Wilcoxon rank-sum test will be used for skewed variables. For time to event data, e.g., time to first

AD episode and time to the first topical corticosteroid exposure, will be calculated using the Kaplan-Meier method. The HRs comparing PAEPAD and standard care will be estimated using cox regression model. Multiple imputation will be conducted if loss to follow-up exceeds 30%. Subgroup analysis will be done for the relative risk of AD, asthma, rhinitis and food sensitization stratified by familial history of atopic disorders, and AD severity for allergic comorbidities (whenever applies). Regression models with interaction terms will be used to test for statistical significance among subgroups.

For sensitivity analysis that shall be done with the PP population, the analyses above will be conducted. For the sensitivity analysis that shall be done with the secondary analysis population, both traditional multivariate comparison and propensity score matching will be used to better balance the covariates and identify comparable groups. An additional sensitivity analysis will be conducted on the population that receives in-person education (as opposed to video recorded).

Data monitoring

An epidemiologist who is independent of the research team will be tasked to monitor the data. An interim analysis on knowledge will be performed when 50% of the patients complete the one-year follow-up. The aim of this interim analysis is to give a better sense if the final primary outcome of incidence will be different between groups, as it is indicated by the stages of change model that knowledge change may lead to behavioral change.

Patient and public involvement

No patient was involved with study design, recruitment or conduct.

Ethics and dissemination

The PAEPAD study is approved by the ethics committee of Capital Medical University Daxing Teaching Hospital. Written informed consent will be obtained from all participants. This study is registered at the Chinese Clinical Trial Registry under the identifier ChiCTR2000040463. Participation in the project is voluntary and will not impact the medical care of the women regardless of their participation status throughout pregnancy. All participants have the right to withdraw from the study at any point and have their data removed from the study database. All patient data will be securely stored and kept accessible by the research members only, with previous authorization from the

PI. The results will be disseminated through peer-reviewed journals. Results will also be communicated at scientific conferences.

DISCUSSION

PAEPAD study is a large single-center randomized controlled trial of an antenatal educational intervention for prevention of atopic dermatitis and the atopic march. Previous studies on educational interventions have been therapeutic, aiming at reducing symptoms and improving quality of life. Therapeutic patient education (secondary prevention) in AD has been proved to be effective with a significant reduction in disease severity[21]. However, little is known about the primary preventive effect of educational and lifestyle interventions.

A strength of this study is the large sample size. In a similar study that aims to evaluate the effect of prenatal education on knowledge and behavioral changes for allergic disease prevention, the sample size was determined based on the behavioral matrices, resulting in a total sample size of 120 [36]. As behavioral changes do not necessarily modify disease outcomes, a larger sample size is warranted to provide adequate power to detect disease outcome differences. Another strength is that we chose the topics of the PAEPAD sessions based on both experts' opinion and a previous survey (Supplementary material, table S2), which indicated that new mothers needed help on infant skin care, safe practice of sun protection and building an unbiased understanding on common topical drugs, especially corticosteroids. We did not discuss about the treatment of atopic diseases further than emphasizing the importance of following the guideline-oriented instructions of physicians [2 13 31-34] at this lecture. Recent pilot studies provided strong efficacy signals for the hypothesis that daily emollient use could prevent atopic dermatitis[15 17]. While the subsequent large trials yielded null results[12 14], it's crucial to realize that the hypotheses in these trials differ significantly. In the pilot studies, emollients were continued until the outcome assessment, whereas in the larger pragmatic trials, a washout period was implemented. In the Barrier Enhancement for Eczema Prevention (BEEP) study, the rates of continued emollient and wash product use extended beyond the intervention period until outcome assessment were as low as four and five percent for the intervention and the control group respectively[12]. Thus, the pilot studies assessed the immediate preventive effect, whereas the larger studies assessed whether this effect, if there is any,

is sustainable. While it's reasonable to hypothesize that the protective effect of emollients may not sustain beyond a year after refraining from application, incorporating conceptual and behavioral changes into daily skincare routine can be substantially beneficial for short term preventive effects.

The current study will explore the preventive effect of an educational intervention, which bears a closer resemblance to the real world scenario through which behavioral changes are achieved. In addition, we plan to longitudinally collect biological specimens to study the crosstalk of lifestyle changes and molecular biology.

The study has some limitations. First, this study is subject to contamination due to the nature of a non-medication intervention and the single-center design. Consequently, the effect size to be detected will likely be a more conservative estimation of the real preventive effect. Therefore, we plan to collect cross-over data and conduct sensitivity analyses based on the PP population. Second, although the PAEPAD lectures will be led by experienced obstetricians and dermatologists, the heterogeneity may nonetheless constitute potential bias. We aim to reduce the heterogeneity by providing a training session and mock classrooms before the project launching during which lecturers will be evaluated on the organization of the class, clarity, student engagement and consistency of performance.

In conclusion, the PAEPAD study will add to our knowledge of the preventive effect of antenatal education on allergic disease outcomes and identify cellular and molecular changes that will warrant future studies. We expect that results from the PAEPAD study will expand our understanding of the primary prevention of allergic disorders.

AUTHORS' CONTRIBUTIONS

Lin Ma and Xiuhua Ma are the principal investigators of this trial and conceptualized the trial. Mutong Zhao, Yuan Liang, Jing Tian, Fengli Song, Ying Wang Lili Ma, Ying Wang, Wanli Gao are responsible for the execution of the project. Mutong Zhao has written the protocol manuscript. Chunping Shen, Shan Wang, Lei Jiao, Yang Wang, Xiaoyan Sun are responsible for data registration. Mutong Zhao and Xiangji Ying will carry out data analyses once the trial is completed. All authors critically reviewed the article. All the authors approved the final manuscript.

Authorship eligibility

Authorship of the consequent publication of this project will be granted to those who made a significant contribution to the conception, design, implementation, analysis of the data, or those who drafted the work or reviewed/revised it critically for important intellectual content.

Availability of data and materials

The datasets to be analyzed will be available from the corresponding author upon reasonable request (requests should be directed to muz880@mail.harvard.edu).

FUNDING

This study has received a public grant from the Capital's Funds for Health Improvement and Research (CFH2020-2-7121). The funders had no role in the design of this study and will not have any role during its execution, analyses, interpretation of data, or submission of outcomes.

COMPETING INTERESTS

None declared.

References

1. Muraro A, Halken S, Arshad SH, et al. EAACI food allergy and anaphylaxis guidelines. Primary prevention of food allergy. *Allergy* 2014;69(5):590-601. doi: 10.1111/all.12398
2. Wollenberg A, Barbarot S, Bieber T, et al. Consensus-based European guidelines for treatment of atopic eczema (atopic dermatitis) in adults and children: part I. *Journal of the European Academy of Dermatology and Venereology : JEADV* 2018;32(5):657-82. doi: 10.1111/jdv.14891
3. Bousquet J, Schunemann HJ, Fonseca J, et al. MACVIA-ARIA Sentinel NetworK for allergic rhinitis (MASK-rhinitis): the new generation guideline implementation. *Allergy* 2015;70(11):1372-92. doi: 10.1111/all.12686
4. Sasaki M, Koplin JJ, Dharmage SC, et al. Prevalence of clinic-defined food allergy in early adolescence: The SchoolNuts study. *The Journal of allergy and clinical immunology* 2018;141(1):391-98 e4. doi: 10.1016/j.jaci.2017.05.041
5. Paller AS, Spergel JM, Mina-Osorio P, et al. The atopic march and atopic multimorbidity: Many trajectories, many pathways. *The Journal of allergy and clinical immunology* 2019;143(1):46-55. doi: 10.1016/j.jaci.2018.11.006
6. Liang Y, Chang C, Lu Q. The Genetics and Epigenetics of Atopic Dermatitis-Filaggrin and Other Polymorphisms. *Clin Rev Allergy Immunol* 2016;51(3):315-28. doi: 10.1007/s12016-015-8508-5 [published Online First: 2015/09/20]
7. Chinthrajah RS, Hernandez JD, Boyd SD, et al. Molecular and cellular mechanisms

of food allergy and food tolerance. *The Journal of allergy and clinical immunology* 2016;137(4):984-97. doi: 10.1016/j.jaci.2016.02.004 [published Online First: 2016/04/10]

8. Shimura S, Takai T, Iida H, et al. Epicutaneous Allergic Sensitization by Cooperation between Allergen Protease Activity and Mechanical Skin Barrier Damage in Mice. *The Journal of investigative dermatology* 2016;136(7):1408-17. doi: 10.1016/j.jid.2016.02.810 [published Online First: 2016/03/19]
9. Guttman-Yassky E, Zhou L, Krueger JG. The skin as an immune organ: Tolerance versus effector responses and applications to food allergy and hypersensitivity reactions. *The Journal of allergy and clinical immunology* 2019;144(2):362-74. doi: 10.1016/j.jaci.2019.03.021 [published Online First: 2019/04/08]
10. Chan SM, Turcanu V, Stephens AC, et al. Cutaneous lymphocyte antigen and alpha4beta7 T-lymphocyte responses are associated with peanut allergy and tolerance in children. *Allergy* 2012;67(3):336-42. doi: 10.1111/j.1398-9995.2011.02765.x [published Online First: 2012/03/17]
11. Deckers J, Bougarne N, Mylka V, et al. Co-Activation of Glucocorticoid Receptor and Peroxisome Proliferator-Activated Receptor-gamma in Murine Skin Prevents Worsening of Atopic March. *The Journal of investigative dermatology* 2018;138(6):1360-70. doi: 10.1016/j.jid.2017.12.023
12. Chalmers JR, Haines RH, Bradshaw LE, et al. Daily emollient during infancy for prevention of eczema: the BEEP randomised controlled trial. *Lancet* 2020;395(10228):962-72. doi: 10.1016/S0140-6736(19)32984-8 [published Online First: 2020/02/23]
13. Eichenfield LF, Tom WL, Berger TG, et al. Guidelines of care for the management of atopic dermatitis: section 2. Management and treatment of atopic dermatitis with topical therapies. *Journal of the American Academy of Dermatology* 2014;71(1):116-32. doi: 10.1016/j.jaad.2014.03.023 [published Online First: 2014/05/13]
14. Skjerven HO, Rehbinder EM, Vettukattil R, et al. Skin emollient and early complementary feeding to prevent infant atopic dermatitis (PreventADALL): a factorial, multicentre, cluster-randomised trial. *Lancet* 2020;395(10228):951-61. doi: 10.1016/S0140-6736(19)32983-6 [published Online First: 2020/02/23]
15. Horimukai K, Morita K, Narita M, et al. Application of moisturizer to neonates prevents development of atopic dermatitis. *The Journal of allergy and clinical immunology* 2014;134(4):824-30 e6. doi: 10.1016/j.jaci.2014.07.060
16. McClanahan D, Wong A, Kezic S, et al. A randomized controlled trial of an emollient with ceramide and filaggrin-associated amino acids for the primary prevention of atopic dermatitis in high-risk infants. *Journal of the European Academy of Dermatology and Venereology : JEADV* 2019;33(11):2087-94. doi: 10.1111/jdv.15786 [published Online First: 2019/07/10]
17. Simpson EL, Chalmers JR, Hanifin JM, et al. Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention. *The Journal of allergy and clinical immunology* 2014;134(4):818-23. doi: 10.1016/j.jaci.2014.08.005

18. Health behavior and health education: Theory, research, and practice, 4th ed. San Francisco, CA, US: Jossey-Bass 2008.
19. World Health Organization ROftEM. Health education: theoretical concepts, effective strategies and core competencies: a foundation document to guide capacity development of health educators, 2012.
20. Tan X, Feldman SR, Chang J, et al. Topical drug delivery systems in dermatology: a review of patient adherence issues. *Expert opinion on drug delivery* 2012;9(10):1263-71. doi: 10.1517/17425247.2012.711756
21. Zhao M, Liang Y, Shen C, et al. Patient Education Programs in Pediatric Atopic Dermatitis: A Systematic Review of Randomized Controlled Trials and Meta-Analysis. *Dermatol Ther (Heidelb)* 2020;10(3):449-64. doi: 10.1007/s13555-020-00365-z [published Online First: 2020/03/23]
22. Sidbury R, Tom WL, Bergman JN, et al. Guidelines of care for the management of atopic dermatitis: Section 4. Prevention of disease flares and use of adjunctive therapies and approaches. *Journal of the American Academy of Dermatology* 2014;71(6):1218-33. doi: 10.1016/j.jaad.2014.08.038
23. Al-Ateeq MA, Al-Rusaiess AA. Health education during antenatal care: the need for more. *International journal of women's health* 2015;7:239-42. doi: 10.2147/IJWH.S75164
24. Parat S, Negre V, Baptiste A, et al. Prenatal education of overweight or obese pregnant women to prevent childhood overweight (the ETOIG study): an open-label, randomized controlled trial. *International journal of obesity* 2019;43(2):362-73. doi: 10.1038/s41366-018-0205-z
25. Suto M, Takehara K, Yamane Y, et al. Effects of prenatal childbirth education for partners of pregnant women on paternal postnatal mental health and couple relationship: A systematic review. *J Affect Disord* 2017;210:115-21. doi: 10.1016/j.jad.2016.12.025 [published Online First: 2016/12/27]
26. Nnam NM. Improving maternal nutrition for better pregnancy outcomes. *Proc Nutr Soc* 2015;74(4):454-9. doi: 10.1017/S0029665115002396 [published Online First: 2015/08/13]
27. Basra MK, Gada V, Ungaro S, et al. Infants' Dermatitis Quality of Life Index: a decade of experience of validation and clinical application. *The British journal of dermatology* 2013;169(4):760-8. doi: 10.1111/bjd.12563 [published Online First: 2013/08/06]
28. Eichenfield LF, Tom WL, Chamlin SL, et al. Guidelines of care for the management of atopic dermatitis: section 1. Diagnosis and assessment of atopic dermatitis. *Journal of the American Academy of Dermatology* 2014;70(2):338-51. doi: 10.1016/j.jaad.2013.10.010 [published Online First: 2013/12/03]
29. Mochizuki H, Kusuda S, Okada K, et al. Palivizumab Prophylaxis in Preterm Infants and Subsequent Recurrent Wheezing. Six-Year Follow-up Study. *Am J Respir Crit Care Med* 2017;196(1):29-38. doi: 10.1164/rccm.201609-1812OC [published Online First: 2017/02/06]
30. Ballardini N, Bergstrom A, Wahlgren CF, et al. IgE antibodies in relation to prevalence and multimorbidity of eczema, asthma, and rhinitis from birth to adolescence. *Allergy* 2016;71(3):342-9. doi: 10.1111/all.12798 [published Online First: 2015/10/28]

31. Wang H, Ma L, Tan Q, et al. Chinese Expert Consensus on the Diagnosis and Management of Food Allergy in Children With Atopic Dermatitis#. 2020;3(3):135-41. doi: 10.1097/jd9.0000000000000091
32. Okubo K, Kurono Y, Ichimura K, et al. Japanese guidelines for allergic rhinitis 2017. *Allergol Int* 2017;66(2):205-19. doi: 10.1016/j.alit.2016.11.001 [published Online First: 2017/02/19]
33. Scadding GK, Kariyawasam HH, Scadding G, et al. BSACI guideline for the diagnosis and management of allergic and non-allergic rhinitis (Revised Edition 2017; First edition 2007). *Clin Exp Allergy* 2017;47(7):856-89. doi: 10.1111/cea.12953 [published Online First: 2017/07/01]
34. Arakawa H, Hamasaki Y, Kohno Y, et al. Japanese guidelines for childhood asthma 2017. *Allergol Int* 2017;66(2):190-204. doi: 10.1016/j.alit.2016.11.003 [published Online First: 2017/01/22]
35. Guo Y, Li P, Tang J, et al. Prevalence of Atopic Dermatitis in Chinese Children aged 1-7 ys. *Scientific reports* 2016;6:29751. doi: 10.1038/srep29751
36. Nakamura R, Ishiguro N, Naru E, et al. The effect of an educational program for pregnant women to prevent allergic diseases in infants: study protocol for a randomized controlled trial. *Trials* 2019;20(1):755. doi: 10.1186/s13063-019-3797-2 [published Online First: 2019/12/23]

Table 1. Outcome assessments of the PAEPAD study

Outcomes	Instruments/Diagnostic criteria	Immediately post intervention	Upon discharge from OB ward	Upon disease flares	3 months postnatal	1 year postnatal	2 year postnatal
Primary outcomes							
Cumulative incidence of AD at 2 years	Hanifin&Rajak						×
Secondary outcomes							
AD outcomes							
Time to first AD episode	Hanifin&Rajak			×			
Time to first topical corticosteroid exposure				×			
Disease related quality of life	IDQOL			×			
Disease severity	SCORAD, EASI and IGA			×			
Frequency of AD flares							
AD disease free days	IGA						
Cumulative clinical visit duration	From the parents being seated within the consulting room to their exit thereafter			×			
Atopic march outcomes							
Asthma incidence							×
Recurrent wheeze incidence							×
Rhinitis incidence							×

Food sensitization incidence	Allergen specific IgE						×
Obstetric outcomes							
Mode of birth	Eutocic/ dystocic/C-section		×				
Pathological pregnancy	Gestational diabetes, pre-eclampsia, placenta previa, placental abruption, prelabor rupture of membranes, puerperal infection, postpartum hemorrhage		×				
Neonatal outcomes:							
Newborn's weight			×				
Admission in neonatal care unit (yes/no).			×				
Apgar score			×				
Fetal growth retardation			×				
Any morbidity of the newborn that results in hospitalization in the first three month of life					×		
Emollient usage	Total volume, brand, frequency				×	×	×
Bathing practice	Frequency, duration of bathing, bath temperature, cleanser usage, soap usage				×	×	×
Patient disease knowledge and attitude changes	Questionnaire currently under validation	×		×	×	×	×

AD, atopic dermatitis; OB, obstetrics; IDQOL, Infantile Dermatitis Quality Of Life; SCORAD, SCORing of Atopic Dermatitis; EASI, Eczema Area and Severity Index; IGA, Investigator Global Assessment

576

577

Table 2. Biological sample collection at fixed follow-up visits

Samples	GW12	GW24-28	GW36	Delivery	24-72h post-partum	42 days post-partum	3 months post-partum	6 months post-partum	12 months post-partum	24 months post-partum
Biological sample of the infant										
Blood				×					×	×
Stool					×	×	×	×	×	×
Skin swab					×	×	×		×	×
Tongue dorsum swab				×	×	×	×	×	×	×
Tape stripping				×	×	×	×		×	×
Biological sample of the mother										
Blood		×	×	×	×					
Stool	×	×	×			×	×			
Urine	×	×	×		×	×	×		×	×
Skin swab		×	×		×	×	×			
Breast milk					×	×	×			
Placenta				×						
Tongue dorsum swab		×	×	×	×	×	×			
Vaginal swab		×	×		×					

GW, gestational week

578

579 Figure legend

580 Fig.1 Flow diagram of the PAEPAD (Preventive Antenatal Educational Program on Allergic

581 Diseases) study.

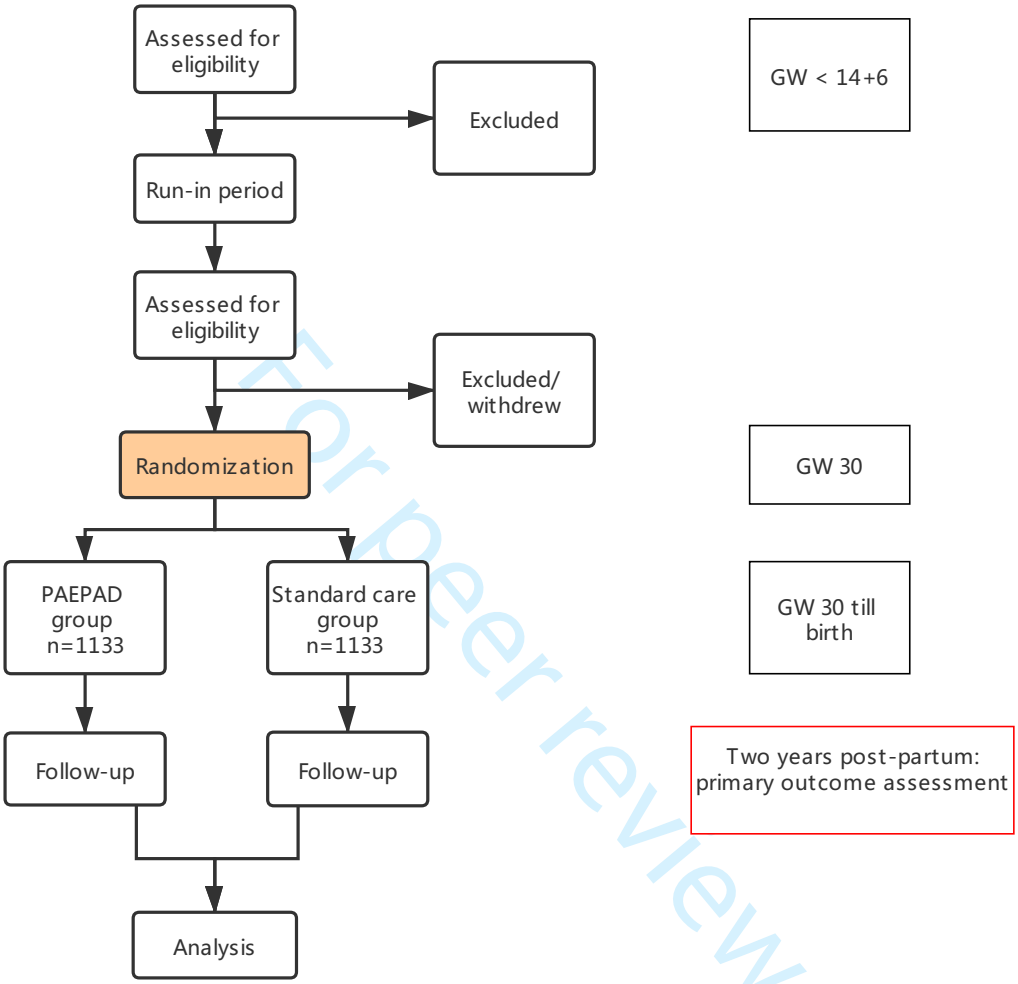

Table of Contents

BIOLOGICAL SAMPLE COLLECTION 2

TABLE S1. RESPONSES OF A 12-ITEM KNOWLEDGE SURVEY 7

TABLE S2. SPECIFIC RECOMMENDATIONS ON BABY SKIN CARE PRACTICES 6

BIOLOGICAL SAMPLE COLLECTION

Blood samples

Blood samples will be collected for analyses of DNA, protein, and exosomes and isolation of Peripheral Blood Mononuclear Cells (PBMCs). The mothers will be asked to be fasting for 8 h or more before the blood draw. Blood samples will be kept at room temperature for 30 min before centrifugation. For DNA analyses, a blood sample of 2ml will be collected at gestational week 24-28 into EDTA tubes and stored at -80°C until use. For protein analyses, the serum will be centrifuged at 1000g for 15 min at room temperature and the supernatants will be aliquoted and stored at − 80 °C until use. For PBMC isolation, PBMC will be purified as previously described, and stored in liquid nitrogen for use [1]. For exosome analyses, 5ml of blood will be collected into EDTA tubes and stored at 4°C for 4 h or overnight. Plasma will be separated by centrifugation at 5000×g for 5 minutes at 4°C. Cell-free, platelet poor plasma will be collected, aliquoted and stored at − 80 °C until use[2].

At birth, the venous and arterial cord blood will be collected from the umbilical cord into EDTA tubes using a syringe. Blood components will be processed and stored as detailed above for analyses of DNA, protein and exosomes. An additional 3mL arterial cord blood will be drawn and stored at room temperature for flow cytometric analysis.

At the year of two, children will be asked to test for serum specific allergens including house dust mite, cat dander, birch pollen, grass pollen, milk, and egg. If the parent consent to having their children's blood drawn, 2ml blood will be collected into tubes containing clot activator and centrifuged at 1000g for 15 min. The supernatant will be aliquoted into 500ul aliquotes and stored at − 80 °C until use.

Urine samples

The first catch midstream urine of mothers will be collected using a sterile cup, centrifuged and added to sterile tubes and stored at –80 °C until analyzed.

Placenta

After delivery, the placenta will be sampled for analyses of histology, DNA, methylation, RNA and protein. First, a cross-section 2cm away from the cord insertion will be sampled and stored in formalin to be fixed for histological examination. Next, twenty pieces of villous tissue on the maternal aspect measuring 3*3*3mm will be taken at 2-4cm away from cord insertion and stored in five cryo-tubes, among which two will be prefilled with RNAlater. Villous samples will be snap-frozen in liquid nitrogen and then stored at -80°C. Third, villous tissue on the fetal aspect will be sampled and stored likewise.

Breast milk

Breast milk will be collected with a breast pump at locations where postnatal follow-ups take place. At follow-up, 30ml of breast milk will be collected with sterilized RNase-free tubes, with the first 500ul disposed. For exosome analysis, 15 ml breast milk will be centrifuged at 4°C for 10 minutes at 1500 g to remove cells and the cream layer. The supernatant will then be transferred to new tubes and centrifuged again at 12,000 g at 4°C to remove remaining cells and cream. The supernatant will then be immediately processed or frozen at -80 °C until use[3]. The remaining 15 ml breast milk sample will be aliquoted and stored at -80 °C directly until use.

Feces

The fecal samples will be self-collected by participants. For 16s RNA sequencing, participants will be provided with a sterile feces collection and preservation kit (ML-001A, Shenzhen Dayun Gene Technology Co., Shenzhen, China). For metabolomics analyses, participants will be provided with a sterile feces collection device with a spatula. Participants will be instructed to collect stool specimens of approximately 10ml within 2 h before each visit and bring them to the visits. Collected feces will then be snap-frozen in liquid nitrogen and stored at -80 °C until use [4]. Meconium of 2ml will be sampled and stored likewise.

Vagina swab

Vaginal swabs (4520CA, COPAN Flock Technologies, Brescia, Italy) will be collected from the posterior fornix by obstetricians. A speculum will be placed in the absence of lubrication. All specimens will be collected by swirling a sterile swab for 30 seconds, withdrawing the swab without contamination from other sites, and transferring the specimen into a sterile tube and stored at -80 °C until use.

Skin swab

Skin specimens will be collected from the cheek, anterior forearm, and lesional sites of the children. The forehead, anterior forearm, and cubital fossa of the mother will be sampled likewise. These sites will be swiped vigorously for 50 times over an area of 4 cm2 using a sterile swab (4520CA, COPAN Flock Technologies, Brescia, Italy). The swab will be premoistened with DNA free saline. Swab specimens will be inserted into a sterile tube and stored at -80 °C until use.

Tape stripping

For each child, skin lipids will be collected by tape stripping from the same body sites as to where skin swabs are sampled from the children. The first layer will be discarded, and the second to fourth layers at the same place will be retained and placed separately in a glass tube

with 5 mL of methanol, then stored at -80 °C until use. The vernix will be sampled from the back of the newborn likewise.

Tongue dorsum swab

Subjects will be instructed not to eat, drink (except water), or brush their tongue during the 12 h period before sampling and not to brush their teeth during the 2 h period before sampling. Specimens will be collected from the central part of the tongue dorsum by swiping for 15 seconds using a sterile swab (4520CA, COPAN Flock Technologies, Brescia, Italy).

References

1. Riedhammer C, Halbritter D, Weissert R. Peripheral Blood Mononuclear Cells: Isolation, Freezing, Thawing, and Culture. *Methods Mol Biol* 2016;1304:53-61. doi: 10.1007/7651_2014_99 [published Online First: 2014/08/06]
2. Stranska R, Gysbrechts L, Wouters J, et al. Comparison of membrane affinity-based method with size-exclusion chromatography for isolation of exosome-like vesicles from human plasma. *J Transl Med* 2018;16(1):1. doi: 10.1186/s12967-017-1374-6 [published Online First: 2018/01/11]
3. Zonneveld MI, Brisson AR, van Herwijnen MJ, et al. Recovery of extracellular vesicles from human breast milk is influenced by sample collection and vesicle isolation procedures. *J Extracell Vesicles* 2014;3 doi: 10.3402/jev.v3.24215 [published Online First: 2014/09/11]
4. Costea PI, Zeller G, Sunagawa S, et al. Towards standards for human fecal sample processing in metagenomic studies. *Nature biotechnology* 2017;35(11):1069-76. doi: 10.1038/nbt.3960

Table S1. Specific Recommendations on Baby Skincare Practices

Part 1. Bathing and Moisturising	
1	The duration of a baby bath should not exceed 10 minutes.
2	Babies should be bathed no less than twice per week and more frequently during summer to reduce sweet irritation.
3	Liquid cleansers with a neutral or mildly acidic pH could be used. Soap-based cleansers should be avoided.
4	Scrubbing and exfoliation should be avoided.
5	Bath temperature should be set to 38-40 °C for newborns and <38 °C for infants.
6	Emollients should be used after bathing, preferably within 5 minutes.
7	Emollients could be used liberally at multiple times to alleviate xerosis.
8	Liquid cleansers and emollients should be fragrance and dye free.
9	Emollients are recommended to be used for the whole body
10	Lotions are recommended for use in hot summer months whereas creams are recommended for use in winter months.
Part 2. Sun Protection	
1	Sun protection A-B-C: Avoid the sun between 10am and 4pm; Block harmful sun rays with a broad-spectrum sunscreen; Cover up with clothing and sunglasses
2	Sunscreen can be used for infants older than 6 months.
Part 3. Commonly Used Topical Agents During Infancy	
1	Topical corticosteroids are the first line therapy for eczema.
2	Topical corticosteroids do not induce premature puberty.
3	Topical corticosteroids used properly will not cause weight gain.
4	Topical corticosteroids used properly will not cause osteoporosis nor hamper the physical development of a child.
5	Prolonged use of topical corticosteroids is associated with higher risk of skin infection, excessive hair growth and skin redness.
6	Topical antibiotics and astringents can be used in eczematic skin.
7	Topical corticosteroids used properly will not cause addiction nor resistence.
Part 4. Atopic dermatitis and Atopic March: An Overview	
1	Atopic dermatitis is one of the most common skin condition during infancy.
2	Significant itch and extensive skin lesions may impose substantial disease burden on the affected child and family.
3	Barrier repair with emollients is the mainstay of treatment for atopic dermatitis.
4	Avoidance of triggers and control of inflammation and infection should be implimented following physician's instructions.
5	Topical corticosteroids are the first line therapy for control opf inflammation in eczematic children.
6	Children with atopic dermatitis are at greater risk for developing othe atopic disorders.

Table S2. Responses of a 12-item Knowledge survey

Survey questions	Score	Number answered (n=395), n (%)
1. I should apply body wash every time I bathe my baby.	1	17 (4.30)
	2	61 (15.44)
	3	61 (15.44)
	4	124 (31.39)
	5	132 (33.42)
2. Moisturizer should be applied all over my baby's body.	1	27(6.84)
	2	86(21.77)
	3	76(19.24)
	4	128(32.41)
	5	78(19.75)
3. Moisturizer should be used no more than once a day.	1	55 (13.92)
	2	109 (27.59)
	3	121 (30.63)
	4	91 (23.04)
	5	19 (4.81)
4. I only use moisturizer after I bathe my baby.	1	25(6.33)
	2	65(16.46)
	3	79(20.00)
	4	170(43.04)
	5	56(14.18)
5. Massage oils can be used as a baby moisturizer.	1	32 (8.10)
	2	96 (24.30)
	3	112 (28.35)
	4	112 (28.35)
	5	43 (10.89)
6. Moisturizer is not necessary for my baby at summer time.	1	10(2.53)
	2	36(9.11)
	3	72(18.23)
	4	160(40.51)
	5	117(29.62)
7. Sweat is an irritant to the baby's skin.	1	39 (9.87)
	2	54 (13.67)
	3	95 (24.5)

	4	105 (26.58)
	5	102 (25.82)
8. I'm concerned to use topical corticosteroids ointment on my baby.	1	103(26.08)
	2	81(20.51)
	3	149(37.72)
	4	37(9.37)
	5	25(6.33)
9. Topical corticosteroids will make the baby fat.	1	43(10.89)
	2	53(13.42)
	3	124(31.39)
	4	105(26.58)
	5	70(17.72)
10. Topical corticosteroids will induce premature puberty in babies.	1	69(17.47)
	2	47(11.9)
	3	138(34.94)
	4	88(22.28)
	5	53(13.42)
11. If I had to use topical corticosteroids on my baby, I'd be concerned that my baby will become addicted to the drug.	1	93(23.54)
	2	67(16.96)
	3	140(35.44)
	4	59(14.94)
	5	36(9.11)
12. If I had to use topical corticosteroids on my baby, I'd be concerned that my baby will become resistant to the drug.	1	88(22.28)
	2	89(22.53)
	3	135(34.18)
	4	57(14.43)
	5	26(6.58)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Page No.
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2, Line 51
	2b	All items from the World Health Organization Trial Registration Data Set	Refer to the appendix
Protocol version	3	Date and version identifier	6, Line 159
Funding	4	Sources and types of financial, material, and other support	15, Line 464
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	14 Line 451
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14 Line 451
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	10 Line 305
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3 Line 72, supplementary table S2
	6b	Explanation for choice of comparators	5 Line 134
Objectives	7	Specific objectives or hypotheses	5 Line 138
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5 Line 148
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6 Line 155
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6 Line 185
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8 Line 220
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	Non pharmacological intervention. NA.
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	8 Line 223-225, line 246-248
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8 Line 244-245

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9 Line 264
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Fig.1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11 Line 330
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6 Line 162
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	7 Line 205
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	7 Line 205
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7 Line 205, 6 line 166, 7 line 212

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7 Line 214
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Non pharmacological intervention. NA.
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10 Line 306
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	9 Line 288
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	9 Line 288
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11 Line 338
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11 Line 360
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11 Line 364
Methods: Monitoring			

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12 Line 370
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12 Line 372
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Non pharmacological intervention. NA.
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Not reported
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	12 Line 380
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6 Line 169
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	6 Line 170
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	10 Line 321

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	14 Line 470
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10 Line 322, 12 Line 386
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	None
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12 Line 388
	31b	Authorship eligibility guidelines and any intended use of professional writers	14 Line 456
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not reported
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Consent form in Chinese are available upon request
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	10 Line 325, supplementary material

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

Appendix. World Health Organization Trial Registration Data Set

Data category	Information
Primary registry and trial identifying number	ChiCTR registry (Trial ID: ChiCTR2000040463)
Date of registration in primary registry	28 November, 2020
Secondary identifying numbers	CFH2020-2-7121
Source(s) of monetary or material support	Capital's Funds for Health Improvement and Research
Primary sponsor	Capital's Funds for Health Improvement and Research
Secondary sponsor(s)	NA
Contact for public queries	Mutong Zhao, MD, MSc. [+86 18600116126r] [muz880@mail.harvard.edu] Lin Ma, MD, Department of Dermatology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China. E-mail: bch_maleen@aliyun.com. Tel: +86 13601305676
Contact for scientific queries	Xiuhua Ma, MD, Department of Obstetrics and Gynecology, Beijing Daxing District People's Hospital, Capital Medical University Daxing Teaching Hospital, Beijing, China. E-mail: mxhdxqyy@126.com Tel: +86 13381021859
Public title	Preventive Antenatal Educational Program on Allergic Diseases (PAEPAD) for prevention of atopic dermatitis
Scientific title	Preventive Antenatal Educational Program on Allergic Diseases (PAEPAD) versus standard antenatal care for prevention of atopic dermatitis: study protocol for a single-center, investigator blinded randomized controlled trial.
Countries of recruitment	China
Health condition(s) or problem(s) studied	Atopic dermatitis
Intervention(s)	PAEPAD group: multi-disciplinary education of neonatal care Standard care group: standard education of neonatal care
Key inclusion and exclusion criteria	Ages eligible for study: ≥18 years Sexes eligible for study: expecting mothers Accepts healthy volunteers: no Inclusion criteria: 1. Enlisted in the birth registry of Daxing Teaching Hospital of Capital Medical University and intend to give birth at this location; 2. Women aged ≥18; 3. Less than 14+6 gestational week when recruited as measured by last menstrual period; 4. Residents of

	Daxing and intend to remain residing in Daxing for at least two years postpartum; 5. Written consent form. Exclusion criteria: 1. Planned abortion; 2. Rare comorbidities of the mother that may cause miscarriage and congenital disabilities as determined by obstetricians, including but not limited to malignancies, congenital heart diseases, monogenetic diseases; 3. Recurrent miscarriage.
Study type	Interventional Allocation: randomized Intervention model: parallel assignment Masking: investigator blind Primary purpose: prevention
Date of first enrolment	30 November, 2020
Target sample size	2266
Recruitment status	Recruiting
Primary outcome(s)	Cumulative incidence of atopic dermatitis at 2 years
Key secondary outcomes	Atopic dermatitis outcomes, atopic march outcomes, obstetric outcomes, neonatal outcomes, parental knowledge outcomes as detailed in the manuscripts
Ethics review	Approved on 23 November, 2020 by the ethical committee of Capital Medical University Daxing Teaching Hospital, Beijing, China.
Completion date	Last subject, last visit
Summary results	NA
IPD sharing statement	IPD will be shared with upon request (requests should be directed to muz880@mail.harvard.edu) for individual data meta-analyses (time frame: 1 year after publication. No end date.).