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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.

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3 1 **TITLE PAGE**

4 2 **Preventive Antenatal Educational Program on Allergic Diseases (PAEPAD) versus**
5 3 **standard antenatal care for prevention of atopic dermatitis: study protocol for a**
6 4 **single-center, investigator-blinded randomized controlled trial.**

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25 **ABSTRACT**

26 **Introduction**

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

32 **Methods and analysis**

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

47 **Ethics and dissemination**

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

51 **Trial registration**

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

54 **Strengths and limitations of the study**

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

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3 56 First attempt to investigate education for primary prevention of incident cases of allergic
4 57 diseases (atopic dermatitis for primary outcome).

5 58 Prone to potential contamination due to single-center design and non-medication
6 59 intervention.

7 60 Potential heterogeneity of intervention effect due to variation in how the intervention will
8 61 be carried out by individual educator albeit provision of formal training for educators.

62 **KEYWORDS**

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

65 **INTRODUCTION**

66 Atopic disorders place a substantial burden on both individuals and the health care
67 system¹⁻⁴. The sequential occurrence of AD, followed by one or more disorders
68 characterized by allergen-specific type 2 (including TH2) responses are designated as
69 atopic march⁵. The underlying mechanisms feature both a genetic susceptibility in which
70 barrier dysfunction and immune dysregulation predispose atopic individuals to Th2
71 immunity, and a progression in which AD and its pathological changes function as
72 instigating events to subsequent development of other atopic comorbidities^{5 6}. The human
73 skin is a functional immune organ with an abundance of immunocompetent cells. In the
74 resting state, an intact skin barrier and a balance of immune cell populations, cytokines,
75 and chemokines promote immune tolerance, which is otherwise disrupted in AD patients.
76 Barrier dysfunction from both loss-of-function mutations in filaggrin and the itch-scratch
77 cycle was postulated as the driving component for atopic march programming by
78 promoting Th2 and Th17 differentiation⁷⁻⁹. Subsequently, sensitizations to food allergens
79 develop in the setting of Th2 skewing, which is otherwise Th1 skewed in the
80 gastrointestinal-homing T cells in tolerant subjects¹⁰. Similarly, in asthmatic airways,
81 disrupted junctional adhesion, mucus plugging develops due to Th2 polarization. While
82 the severity of subsequent allergic airway inflammation was affected by AD, abrogation
83 of AD by topical treatment prevents worsening of subsequent airway inflammation by
84 counteracting the Th17 pathway¹¹. Granted that both the genetic predisposition and the
85 atopic progression contribute to the development of atopic march, longitudinal studies are
86 warranted to explore the impact of immune activation of AD on the development of other

1
2
3 87 TH2 comorbidities. Meanwhile, the role of epithelial-immune crosstalk in the atopic
4
5 88 march needs to be further elucidated.
6
7 89 To date, extensive research has been conducted on the therapeutic effect of barrier
8
9 90 enhancement using emollients. Compelling evidence indicated that emollients render the
10
11 91 skin less susceptible to irritants and reduce flares of AD (secondary prevention)¹² and
12
13 92 therefore was regarded as a cornerstone of AD therapy¹³. However, evidence was
14
15 93 inconsistent regarding the preventive effect of emollient application^{12 14-17}. The
16
17 94 discordance from these behavioral intervention studies is probably a result of the
18
19 95 difference in when the outcome assessment took place. In the BEEP¹² and
20
21 96 PreventADALL¹⁴ studies, the outcome was measured after a long washout period to
22
23 97 ensure that any mild AD would not be concealed by the on-going application of
24
25 98 emollients, whereas in the small studies^{15 16} the immediate effect was evaluated.
26
27 99 However, the null long-term result should not overshadow the strong efficacy signal
28
29 100 displayed at the immediate outcome assessments. Moreover, as behavior change is a
30
31 101 process that unfolds through a continuum of stages from pre-contemplation,
32
33 102 contemplation, preparation, to action and maintenance, such straightforward behavioral
34
35 103 intervention may not echo with the real-world scenario, where behavior changes are
36
37 104 achieved through health promotion initiatives, including health education and health
38
39 105 policies^{18 19}. Consequently, the generalizability of these studies might be affected due to
40
41 106 the difference in how intervention was delivered and the setting under which outcome
42
43 107 assessment took place.
44
45 108 In realizing the tremendous burden inflicted by atopic disorders and the critical role of
46
47 109 AD in atopic march, significant therapeutic discoveries have been made over the past
48
49 110 century. Nonetheless, in AD and other chronic diseases alike, adherence to treatment can
50
51 111 be strikingly poor²⁰. The underlying factors were postulated to be the complexity of
52
53 112 treatment regimen, corticosteroid phobia, and caregiver burden²⁰. Addressing these
54
55 113 issues calls for consistent efforts in patient education that can hardly be accomplished in a
56
57 114 typical clinical visit. Recent studies have shown that structured patient education can
58
59 115 significantly reduce disease severity in AD patients²¹. Given this, guidelines for AD have
60
116 acknowledged education of patients and caregivers as an essential form of secondary
117 prevention to reduce disease flares and improve quality of lives²². However, little is

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2
3 118 known about how education performs as a primary prevention approach to reduce AD
4
5 119 disease burden.
6
7 120 Antenatal education is a crucial component of antenatal care. Since its implementation
8
9 121 from the nineteenth century, mortality in children has decreased tremendously. In 2005,
10
11 122 the World Health Organization called for “realizing the Potential of Antenatal Care”²³,
12
13 123 and thence started using it as a platform for primary prevention of malnutrition,
14
15 124 HIV/AIDS, sexually transmitted infections, tuberculosis, and prevention of postpartum
16
17 125 and neonatal diseases²⁴⁻²⁶. Its diversified usage has led to us hypothesize that educational
18
19 126 intervention delivered through the antenatal education platform may yield informative
20
21 127 data for assessing the primary prevention of allergic diseases. Thus, the Preventive
22
23 128 Antenatal Education Program on Allergic Diseases (PAEPAD) study focuses on using
24
25 129 the antenatal care platform as a more affordable and effective health education approach.
26
27 130 In this investigator-blinded, randomized controlled trial, we aim to evaluate the effect of
28
29 131 such intervention vs. standard education on atopic disease outcomes. The primary
30
31 132 objective is to evaluate the preventive effect of PAEPAD on atopic dermatitis and atopic
32
33 133 comorbidity incidences. The secondary objectives are: 1) to explore the immune-barrier
34
35 134 crosstalk between the immune system, commensal flora and skin barrier function that
36
37 135 may explain the development of atopic march; 2) to identify biomarkers (metabolites,
38
39 136 MicroRNAs) that can be used to characterize individuals at high risk of AD and atopic
40
41 137 march; 3) to elucidate whether and to what extent the maternal immune milieu influences
42
43 138 AD development of the child through cord blood and breast milk.

139 **METHODS AND ANALYSIS**

140 **Study design**

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

1
2
3 148 approximately 2.7% of Beijing's annual deliveries. This protocol was drafted (Aug 17th,
4 149 2020) before participant recruitment (Oct 30th, 2020).

6 150 **RECRUITMENT, INCLUSION AND EXCLUSION CRITERIA**

8 151 All expectant parents living in the catchment area of the Daxing Teaching Hospital of
9 152 Capital Medical University will receive an information leaflet about the study at their
11 153 first visit to the maternity clinic. Recruitment will take place in their first mandatory
13 154 maternity class between gestation week 7 to 14+6. All expectant mothers will be given
15 155 detailed information about the study. A trained research nurse will outline key
16 156 information (i.e., inclusion and exclusion criteria, scheduled research visits, and
18 157 instructions on biological sample collection) about the study in the form of a short lecture
20 158 session. The parents who wish to participate will be requested to provide written
21 159 informed consent. They will be inquired on five levels of consent: 1) consent to
23 160 participate in the study; 2) consent to biological sample collections of the mother that are
24 161 non-invasive, including but not limited to the recollection of blood from routine
26 162 pregnancy workups; 3) consent to biological sample collections of the mother that are
27 163 minimally invasive or could potentially cause discomfort, i.e., additional blood draw and
28 164 vaginal swabs; 4) consent to biological sample collections of the child that are non-
30 165 invasive, including but not limited to the recollection of blood from routine checkups; 5)
31 166 consent to biological sample collections of the child that are minimally invasive or could
32 167 potentially cause discomfort, i.e., additional blood draw and tape stripping. At birth,
33 168 another consent form on the children's biological sample collection will be signed to
34 169 allow for any change of consent status on non-invasive and minimally invasive sample
35 170 collections. Consented participants will subsequently receive a QR code through their
36 171 cellphone after registration. By scanning the QR code, the recruitment staff will be able
37 172 to confirm their participation status, at the end of which a study-specific ID number will
38 173 be generated automatically.

40 174 **Inclusion and exclusion criteria**

41 175 Run-in phase inclusion criteria: 1. Enlisted in the birth registry of Daxing Teaching
42 176 Hospital of Capital Medical University and intend to give birth at this location; 2.
43 177 Women aged ≥ 18 ; 3. Less than 14+6 gestational week when recruited as measured by last

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

180 Randomization phase inclusion criteria:

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

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

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

193 **Treatment allocation**

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

202 **Blinding**

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

207 **Intervention**

208 After randomization at GW 30, the participants will be informed on their allocation, and
209 members of the research team will send out weekly invitations through E-mail to
210 participants who have not yet completed the intervention. For those who failed to complete
211 the intervention prior to admission into the OB department, a pre-recorded video will be
212 played during their hospital stay. In the standard care group, patients will receive the
213 standard neonatal care session from an experienced obstetrician, which will include
214 breastfeeding, newborn screening tests, infant physiology, immunization, solid food
215 introduction, belly and eye care (45 min). This session is one of the five mandatory sessions
216 with participation of 83.7-91.9% over the past five years (unpublished data). The treatment
217 group will receive an educational program designed by a multi-disciplinary group of
218 experienced obstetricians and pediatric dermatologists. The program will be focused
219 primarily on: 1) Standard education of neonatal care as the control group (45 min); 2) Skin
220 care of the newborns with a practical demonstration on bathing and emollient application
221 (20 min); 3) Sun protection (3 min); 4) A brief introduction on commonly used topical
222 agents during infancy, including topical corticosteroids, antibiotics, and astringents (5 min);
223 5) Besides, the program will also contain a 5 minutes presentation on atopic dermatitis
224 disease burden, its precipitators, managements, disease courses, and the atopic march. At
225 the neonatal care class, all participants will first receive the standard education, which will
226 be held concurrently in two separate rooms to minimize group contamination. At the end
227 of the sessions, participants of the PAEPAD cohort will be asked to participate in the
228 PAEPAD session, which will last for less than 40 min. And participants of the standard
229 neonatal care cohort will be asked to leave. Any crossover and non-compliance will be
230 surveyed by a research nurse at the beginning and end of the antenatal sessions. The
231 research nurses who collect data on compliance and who send out invitations will not be
232 involved in outcome assessment.

233 **Study outcomes and follow-up**

234 Study outcomes

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

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2
3 238 Diagnostic criteria for AD will be based on Hanifin&Rajka criteria, which is regarded as
4
5 239 the ‘gold standard’ for hospital-based research. The Infants’ Dermatitis Quality of Life
6
7 240 Index (IDQoL) is a questionnaire of ten items that has been translated into 21 languages.
8
9 241 This questionnaire is validated in infants aged 0–3 years²⁷. Disease severity at disease
10
11 242 flares will be measured by SCORing of Atopic Dermatitis (SCORAD) and Eczema Area
12
13 243 and Severity Index (EASI) and through the course of the disease by Investigator’s Global
14
15 244 Assessment (IGA). SCORAD and EASI are validated instruments for disease severity
16
17 245 assessment²⁸. IGA provides the most straightforward assessment of disease severity and
18
19 246 will thus be employed to assess AD disease-free days. An IGA of less than two is defined
20
21 247 as clearance of disease. An episode of expiratory wheezing will be defined as bronchial
22
23 248 obstruction lasting for at least 24 hours preceded by at least a one-week non-wheezing
24
25 249 healthy period, as defined by a physician. Recurrent wheezing will be defined as the
26
27 250 occurrence of 3 or more episodes of expiratory wheezing diagnosed by a physician in a
28
29 251 12-month period²⁹. Rhinitis will be defined as symptoms of sneezing, a runny or blocked
30
31 252 nose, or itchy, red and watery eyes after exposure to furred pets or pollen the year before
32
33 253 follow-up and/or doctor’s diagnosis of allergic rhinitis³⁰. Sensitization will be defined as
34
35 254 allergen-specific IgE ≥ 0.35 kU_A/l. Parental knowledge and attitude will be quantified
36
37 255 using a questionnaire. The items of the questionnaire were developed following rigorous
38
39 256 procedures, including a review of literature, a patient focus group and a panel discussion
40
41 257 of experts. This questionnaire is currently under validation (unpublished data).
42
43 258 Follow-up
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45 259 Participants will receive both fixed and disease prompted follow-up visits. Follow-up
46
47 260 visits will be carried out at Daxing hospital and five health service centers during
48
49 261 immunization. At follow-ups, biological samples, surveys and physical examination data
50
51 262 will be collected. Specific items are listed in Table. 2. In addition, children will be
52
53 263 assessed upon disease onsets and flares. During follow-ups, all cases will be treated
54
55 264 according to the guidelines^{2 13 31-34} by specialists who are actively engaged in the care of
56
57 265 pediatric AD patients. Participants are not allowed to participate in other clinical trials
58
59 266 after inclusion into the study till the end of the last follow-up visit
60

267 **Covariates**

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

275 **Data collection and management**

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

293 **Biological sample collection**

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

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

299 **Sample size**

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

307 **Analyses**

308 Definition of population sets

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

316 Statistical analysis

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

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

1
2
3 328 30%. Subgroup analysis will be done for the relative risk of AD, asthma, rhinitis and food
4 329 sensitization stratified by familial history of atopic disorders, and AD severity for allergic
5 330 comorbidities (whenever applies). Regression models with interaction terms will be used
6
7 331 to test for statistical significance among subgroups.

8
9 332 For sensitivity analysis that shall be done with the PP population, the analyses above will
10 333 be conducted. For the sensitivity analysis that shall be done with the secondary analysis
11 334 population, both traditional multivariate comparison and propensity score matching will
12 335 be used to better balance the covariates and identify comparable groups. An additional
13 336 sensitivity analysis will be conducted on the population that receives in-person education
14 337 (as opposed to video recorded).

20 338 **Data monitoring**

21 339 An epidemiologist who is independent of the research team will be tasked to monitor the
22 340 data. An interim analysis will be performed when 50% of the patients complete the one-
23 341 year follow-up. The epidemiologist will conclude based on the interim analysis if the
24 342 intervention is proved to be different from the control (standard management) and report
25 343 to the Principal Investigator (PI). The PI can then decide whether or not to modify
26 344 recruitment.

32 345 **Patient and public involvement**

33 346 No patient was involved with study design, recruitment or conduct.

34 347 **Ethics and dissemination**

35 348 The PAEPAD study is approved by the ethics committee of Capital Medical University
36 349 Daxing Teaching Hospital. Written informed consent will be obtained from all
37 350 participants. This study is registered at the Chinese Clinical Trial Registry under the
38 351 identifier ChiCTR2000040463. Participation in the project is voluntary and will not
39 352 impact the medical care of the women regardless of their participation status throughout
40 353 pregnancy. All participants have the right to withdraw from the study at any point and
41 354 have their data removed from the study database. All patient data will be securely stored
42 355 and kept accessible by the research members only, with previous authorization from the
43 356 PI. The results will be disseminated through peer-reviewed journals. Results will also be
44 357 communicated at scientific conferences.

54 358 **DISCUSSION**

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2
3 359 PAEPAD study is a large single-center randomized controlled trial of an antenatal
4 educational intervention for prevention of atopic dermatitis and the atopic march.
5 360
6 361 Previous studies on educational interventions have been therapeutic, aiming at reducing
7 symptoms and improving quality of life. Therapeutic patient education (secondary
8 362 prevention) in AD has been proved to be effective with a significant reduction in disease
9 severity²¹. However, little is known about the primary preventive effect of educational
10 363 and lifestyle interventions.
11
12 364 A strength of this study is the large sample size. In a similar study that aims to evaluate
13 the effect of prenatal education on knowledge and behavioral changes for allergic disease
14 prevention, the sample size was determined based on the behavioral matrices, resulting in
15 365 a total sample size of 120³⁶. As behavioral changes do not necessarily modify disease
16 outcomes, a larger sample size is warranted to provide adequate power to detect disease
17 366 outcome differences. Another strength is that we chose the topics of the PAEPAD
18 sessions based on both experts' opinion and a previous survey (Supplementary material,
19 367 table S1), which indicated that new mothers needed help on infant skin care, safe practice
20 of sun protection and building an unbiased understanding on common topical drugs,
21 especially corticosteroids. We did not discuss about the treatment of atopic diseases
22 368 further than emphasizing the importance of following the instructions of physicians on a
23 guideline-oriented^{2 13 31-34} management at this lecture. Recent pilot studies provided
24 strong efficacy signals for the hypothesis that daily emollient use could prevent atopic
25 369 dermatitis^{15 17}. While the subsequent large trials yielded null results^{12 14}, it's crucial to
26 realize that the hypotheses in these trials differ significantly. In the pilot studies,
27 370 emollients were continued until the subsequent outcome assessment, whereas in the
28 larger pragmatic trials, a washout period was implemented. In the Barrier Enhancement
29 371 for Eczema Prevention (BEEP) study, the rates of continued emollient and wash product
30 use extended beyond the intervention period until outcome assessment were as low as
31 372 four and five percent for the intervention and the control group respectively¹². Thus, the
32 pilot studies assessed the immediate preventive effect, whereas the larger studies assessed
33 373 whether this effect, if there is any, is sustainable. It's reasonable to hypothesize that the
34 protective effect of emollients may not sustain beyond a year after refraining from
35 374 application.
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3 390 The current study will explore the preventive effect of an educational intervention, which
4 391 bears a closer resemblance to the real world scenario through which behavioral changes
5 392 are achieved. In addition, we plan to longitudinally collect biological specimens to study
6 393 the crosstalk of lifestyle changes and molecular biology.

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10 394 The study has some limitations. First, this study is subject to contamination due to the
11 395 nature of a non-medication intervention and the single-center design. Consequently, the
12 396 effect size to be detected will likely be a more conservative estimation of the real
13 397 preventive effect. Therefore, we plan to collect cross-over data and conduct sensitivity
14 398 analyses based on the PP population. Second, although the PAEPAD lectures will be led
15 399 by experienced obstetricians and dermatologists, the heterogeneity may nonetheless
16 400 constitute potential bias. We aim to reduce the heterogeneity by providing a training
17 401 session and mock classrooms before the project launching during which lecturers will be
18 402 evaluated on the organization of the class, clarity, student engagement and consistency of
19 403 performance.

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27 404 In conclusion, the PAEPAD study will add to our knowledge of the preventive effect of
28 405 antenatal education on allergic disease outcomes and identify cellular and molecular
29 406 changes that will warrant future studies. We expect that results from the PAEPAD study
30 407 will expand our understanding of the primary prevention of allergic disorders.

31 408 **AUTHORS' CONTRIBUTIONS**

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36 409 Lin Ma and Xiuhua Ma are the principal investigators of this trial and conceptualized the
37 410 trial. Mutong Zhao, Yuan Liang, Jing Tian, Fengli Song, Ying Wang are responsible for
38 411 the execution of the project. Mutong Zhao has written the first protocol manuscript. All
39 412 authors critically reviewed the article. All the authors approved the final manuscript.

40 413 **Authorship eligibility**

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43
44 414 Authorship of the consequent publication of this project will be granted to those who
45 415 made a significant contribution to the conception, design, implementation, analysis of the
46 416 data, or those who drafted the work or reviewed/revised it critically for important
47 417 intellectual content.

48 418 **Availability of data and materials**

49
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51
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53 419 The datasets to be analyzed will be available from the corresponding author upon
54 420 reasonable request (requests should be directed to muz880@mail.harvard.edu).

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3 421 **FUNDING**

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5 and Research (CFH2020-2-7121). The funders had no role in the design of this study and
6 423
7 will not have any role during its execution, analyses, interpretation of data, or submission
8 424
9 of outcomes.
10 425

11 426 **COMPETING INTERESTS**

12 427 None declared.
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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						×

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4									
5	Rhinitis incidence								×
6	Food sensitization incidence	Allergen specific IgE							×
7									
8	Obstetric outcomes								
9									
10	Mode of birth	Eutocic/ dystocic/C-section							×
11									
12									
13									
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16		Gestational diabetes, pre-eclampsia, placenta							
17		previa, placental							
18		abruption, prelabor							
19		rupture of membranes,							
20		puerperal infection,							
21		postpartum hemorrhage							×
22	Pathological pregnancy								
23	Neonatal outcomes:								
24									
25	Newborn.s weight								×
26									
27	Admission in neonatal care								
28	unit (yes/no).								×
29	Apgar score								×
30	Fetal growth retardation								×
31									
32									
33									
34	Any morbidity of the newborn								
35	that results in hospitalization in								
36	the first three month of life								×
37									
38	Patient disease knowledge and	Questionnaire currently							
39	attitude changes	under validation							×
40			×				×		×
41									
42									
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46									
47									

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

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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		×	×	×	×	×	×			

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Vaginal
swab

×

×

×

GW, gestational week

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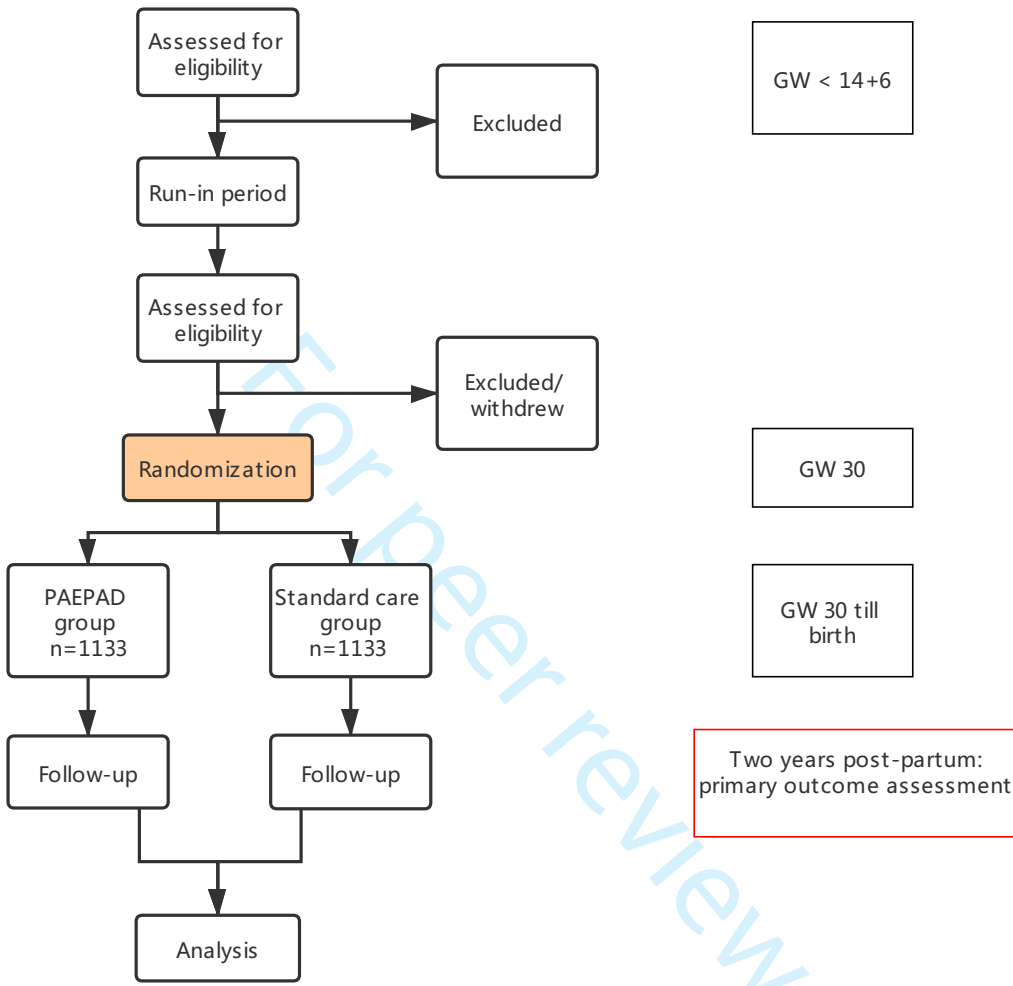
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2
3 567 Figure legend

4 568 Fig.1 Flow diagram of the PAEPAD (Preventive Antenatal Educational Program on Allergic

5 569 Diseases) study.
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3 **Table of Contents**
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6 **BIOLOGICAL SAMPLE COLLECTION2**
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9 **TABLE S1. RESPONSES OF A 12-ITEM KNOWLEDGE SURVEY6**
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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 use¹. 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 use².

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

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3 Breast milk will be collected with a breast pump at locations where postnatal follow-ups take
4 place. At follow-up, 30ml of breast milk will be collected with sterilized RNase-free tubes,
5 with the first 500ul disposed. For exosome analysis, 15 ml breast milk will be centrifuged at
6 4°C for 10 minutes at 1500 g to remove cells and the cream layer. The supernatant will then
7 be transferred to new tubes and centrifuged again at 12,000 g at 4°C to remove remaining
8 cells and cream. The supernatant will then be immediately processed or frozen at -80 °C until
9 use³. The remaining 15 ml breast milk sample will be aliquoted and stored at -80 °C directly
10 until use.
11

12 **Feces**

13 The fecal samples will be self-collected by participants. For 16s RNA sequencing,
14 participants will be provided with a sterile feces collection and preservation kit (ML-001A,
15 Shenzhen Dayun Gene Technology Co., Shenzhen, China). For metabolomics analyses,
16 participants will be provided with a sterile feces collection device with a spatula. Participants
17 will be instructed to collect stool specimens of approximately 10ml within 2 h before each
18 visit and bring them to the visits. Collected feces will then be snap-frozen in liquid nitrogen
19 and stored at -80 °C until use⁴. Meconium of 2ml will be sampled and stored likewise.
20

21 **Vagina swab**

22 Vaginal swabs (4520CA, COPAN Flock Technologies, Brescia, Italy) will be collected from
23 the posterior fornix by obstetricians. A speculum will be placed in the absence of lubrication.
24 All specimens will be collected by swirling a sterile swab for 30 seconds, withdrawing the
25 swab without contamination from other sites, and transferring the specimen into a sterile tube
26 and stored at -80 °C until use.
27

28 **Skin swab**

29 Skin specimens will be collected from the cheek, anterior forearm, and lesional sites of the
30 children. The forehead, anterior forearm, and cubital fossa of the mother will be sampled
31 likewise. These sites will be swiped vigorously for 50 times over an area of 4 cm² using a
32 sterile swab (4520CA, COPAN Flock Technologies, Brescia, Italy). The swab will be
33 premoistened with DNA free saline. Swab specimens will be inserted into a sterile tube and
34 stored at -80 °C until use.
35

36 **Tape stripping**

37 For each child, skin lipids will be collected by tape stripping from the same body sites as to
38 where skin swabs are sampled from the children. The first layer will be discarded, and the
39 second to fourth layers at the same place will be retained and placed separately in a glass tube
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3 with 5 mL of methanol, then stored at -80 °C until use. The vernix will be sampled from the
4 back of the newborn likewise.

5
6 **Tongue dorsum swab**

7
8 Subjects will be instructed not to eat, drink (except water), or brush their tongue during the
9 12 h period before sampling and not to brush their teeth during the 2 h period before
10 sampling. Specimens will be collected from the central part of the tongue dorsum by swiping
11 for 15 seconds using a sterile swab (4520CA, COPAN Flock Technologies, Brescia, Italy).
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Table S1. Responses of a 12-item Knowledge survey

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

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

1				
2	Background and	6a	Description of research question and	3 Line 61
3	rationale		justification for undertaking the trial, including	
4			summary of relevant studies (published and	
5			unpublished) examining benefits and harms	
6			for each intervention	
7				
8		6b	Explanation for choice of comparators	5 Line 125
9				
10	Objectives	7	Specific objectives or hypotheses	5 Line 126
11				
12	Trial design	8	Description of trial design including type of trial	5 Line 125
13			(eg, parallel group, crossover, factorial, single	
14			group), allocation ratio, and framework (eg,	
15			superiority, equivalence, noninferiority,	
16			exploratory)	
17				
18				
19				

Methods: Participants, interventions, and outcomes

20				
21				
22	Study setting	9	Description of study settings (eg, community	5 Line 136
23			clinic, academic hospital) and list of countries	
24			where data will be collected. Reference to	
25			where list of study sites can be obtained	
26				
27	Eligibility criteria	10	Inclusion and exclusion criteria for participants.	6 Line 169
28			If applicable, eligibility criteria for study centres	
29			and individuals who will perform the	
30			interventions (eg, surgeons, psychotherapists)	
31				
32				
33	Interventions	11a	Interventions for each group with sufficient	8 Line 201
34			detail to allow replication, including how and	
35			when they will be administered	
36				
37		11b	Criteria for discontinuing or modifying	Non
38			allocated interventions for a given trial	pharmacological
39			participant (eg, drug dose change in response	intervention.
40			to harms, participant request, or	NA.
41			improving/worsening disease)	
42				
43				
44		11c	Strategies to improve adherence to	8 Line 201- 6
45			intervention protocols, and any procedures for	
46			monitoring adherence (eg, drug tablet return,	
47			laboratory tests)	
48				
49				
50		11d	Relevant concomitant care and interventions	9 Line 260
51			that are permitted or prohibited during the trial	
52				
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2	Outcomes	12	Primary, secondary, and other outcomes,	8 Line 228
3			including the specific measurement variable	
4			(eg, systolic blood pressure), analysis metric	
5			(eg, change from baseline, final value, time to	
6			event), method of aggregation (eg, median,	
7			proportion), and time point for each outcome.	
8			Explanation of the clinical relevance of chosen	
9			efficacy and harm outcomes is strongly	
10			recommended	
11				
12				
13	Participant	13	Time schedule of enrolment, interventions	Fig.1
14	timeline		(including any run-ins and washouts),	
15			assessments, and visits for participants. A	
16			schematic diagram is highly recommended	
17			(see Figure)	
18				
19				
20	Sample size	14	Estimated number of participants needed to	11 Line 294
21			achieve study objectives and how it was	
22			determined, including clinical and statistical	
23			assumptions supporting any sample size	
24			calculations	
25				
26				
27	Recruitment	15	Strategies for achieving adequate participant	6 Line 148
28			enrolment to reach target sample size	
29				

Methods: Assignment of interventions (for controlled trials)

Allocation:

33				
34				
35	Sequence	16a	Method of generating the allocation sequence	7 Line 187
36	generation		(eg, computer-generated random numbers),	
37			and list of any factors for stratification. To	
38			reduce predictability of a random sequence,	
39			details of any planned restriction (eg, blocking)	
40			should be provided in a separate document	
41			that is unavailable to those who enrol	
42			participants or assign interventions	
43				
44				
45	Allocation	16b	Mechanism of implementing the allocation	7 Line 193
46	concealment		sequence (eg, central telephone; sequentially	
47	mechanism		numbered, opaque, sealed envelopes),	
48			describing any steps to conceal the sequence	
49			until interventions are assigned	
50				
51				
52	Implementation	16c	Who will generate the allocation sequence,	7 Line 187
53			who will enrol participants, and who will assign	
54			participants to interventions	
55				
56				
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1				
2	Blinding	17a	Who will be blinded after assignment to	7 Line 196
3	(masking)		interventions (eg, trial participants, care	
4			providers, outcome assessors, data analysts),	
5			and how	
6				
7		17b	If blinded, circumstances under which	Non
8			unblinding is permissible, and procedure for	pharmacological
9			revealing a participant's allocated intervention	intervention.
10			during the trial	NA.
11				
12				

Methods: Data collection, management, and analysis

13				
14				
15	Data collection	18a	Plans for assessment and collection of	8 Line 227
16	methods		outcome, baseline, and other trial data,	
17			including any related processes to promote	
18			data quality (eg, duplicate measurements,	
19			training of assessors) and a description of	
20			study instruments (eg, questionnaires,	
21			laboratory tests) along with their reliability and	
22			validity, if known. Reference to where data	
23			collection forms can be found, if not in the	
24			protocol	
25				
26				
27				
28		18b	Plans to promote participant retention and	10 Line 269
29			complete follow-up, including list of any	
30			outcome data to be collected for participants	
31			who discontinue or deviate from intervention	
32			protocols	
33				
34				
35	Data	19	Plans for data entry, coding, security, and	10 Line 269
36	management		storage, including any related processes to	
37			promote data quality (eg, double data entry;	
38			range checks for data values). Reference to	
39			where details of data management procedures	
40			can be found, if not in the protocol	
41				
42				
43	Statistical	20a	Statistical methods for analysing primary and	14 Line 392
44	methods		secondary outcomes. Reference to where	
45			other details of the statistical analysis plan can	
46			be found, if not in the protocol	
47				
48				
49		20b	Methods for any additional analyses (eg,	14 Line 406
50			subgroup and adjusted analyses)	
51				
52		20c	Definition of analysis population relating to	13 Line 381
53			protocol non-adherence (eg, as randomised	
54			analysis), and any statistical methods to	
55			handle missing data (eg, multiple imputation)	
56				
57				

Methods: Monitoring

1				
2	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
3				
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12		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
13				
14				
15				
16				
17				
18	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.
19				
20				
21				
22				
23				
24	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
25				
26				
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29				
30	Ethics and dissemination			
31				
32	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	12 Line 344
33				
34				
35				
36	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
37				
38				
39				
40				
41				
42				
43	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
44				
45				
46				
47				
48		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	6 Line 153
49				
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51				
52	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
53				
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1				
2	Declaration of	28	Financial and other competing interests for	15 Line 422
3	interests		principal investigators for the overall trial and	
4			each study site	
5				
6	Access to data	29	Statement of who will have access to the final	10 Line 283, 12
7			trial dataset, and disclosure of contractual	Line 350
8			agreements that limit such access for	
9			investigators	
10				
11	Ancillary and	30	Provisions, if any, for ancillary and post-trial	None
12	post-trial care		care, and for compensation to those who	
13			suffer harm from trial participation	
14				
15				
16	Dissemination	31a	Plans for investigators and sponsor to	12 Line 343
17	policy		communicate trial results to participants,	
18			healthcare professionals, the public, and other	
19			relevant groups (eg, via publication, reporting	
20			in results databases, or other data sharing	
21			arrangements), including any publication	
22			restrictions	
23				
24				
25				
26		31b	Authorship eligibility guidelines and any	14 Line 409
27			intended use of professional writers	
28				
29		31c	Plans, if any, for granting public access to the	Not reported
30			full protocol, participant-level dataset, and	
31			statistical code	
32				
33				
34	Appendices			
35				
36	Informed consent	32	Model consent form and other related	Available upon
37	materials		documentation given to participants and	request
38			authorised surrogates	
39				
40	Biological	33	Plans for collection, laboratory evaluation, and	10 Line 287,
41	specimens		storage of biological specimens for genetic or	supplementary
42			molecular analysis in the current trial and for	material
43			future use in ancillary studies, if applicable	
44				

*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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" 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 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
Contact for scientific queries	Preventive Antenatal Educational Program on Allergic Diseases (PAEPAD) for prevention of atopic dermatitis
Public 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.
Scientific title	
Countries of recruitment	China
Health condition(s) or problem(s) studied	Atopic dermatitis PAEPAD group: <i>multi-disciplinary education of neonatal care</i>
Intervention(s)	Standard care group: standard education of neonatal care 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
Key inclusion and exclusion criteria	

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.

Interventional

Allocation: randomized

Intervention model: parallel assignment

Masking: investigator blind

Study type 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

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.).

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:	<i>BMJ Open</i>
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

SCHOLARONE™
Manuscripts

1
2
3 **1 TITLE PAGE**

4 **2 Preventive Antenatal Educational Program on Allergic Diseases (PAEPAD) versus**
5 **3 standard antenatal care for prevention of atopic dermatitis: study protocol for a**
6 **4 single-center, investigator-blinded randomized controlled trial.**

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Word count: 4407

25 **ABSTRACT**

26 **Introduction**

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

32 **Methods and analysis**

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

52 **Ethics and dissemination**

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

56 **Trial registration**

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

59 **Strengths and limitations of the study**

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

68 **KEYWORDS**

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

71 **INTRODUCTION**

72 Atopic disorders place a substantial burden on both individuals and the health care
73 system¹⁻⁴. The sequential occurrence of AD, followed by one or more disorders
74 characterized by allergen-specific type 2 (including TH2) responses are designated as
75 atopic march⁵. The underlying mechanisms feature both a genetic susceptibility in which
76 barrier dysfunction and immune dysregulation predispose atopic individuals to Th2
77 immunity, and a progression in which AD and its pathological changes function as
78 instigating events to subsequent development of other atopic comorbidities^{5 6}. The human
79 skin is a functional immune organ with an abundance of immunocompetent cells. In the
80 resting state, an intact skin barrier and a balance of immune cell populations, cytokines,
81 and chemokines promote immune tolerance, which is otherwise disrupted in AD patients.
82 Barrier dysfunction from both loss-of-function mutations in filaggrin and the itch-scratch
83 cycle was postulated as the driving component for atopic march programming by
84 promoting Th2 and Th17 differentiation⁷⁻⁹. Subsequently, sensitizations to food allergens
85 develop in the setting of Th2 skewing, which is otherwise Th1 skewed in the
86 gastrointestinal-homing T cells in tolerant subjects¹⁰. Similarly, in asthmatic airways,

1
2
3 87 disrupted junctional adhesion, mucus plugging develops due to Th2 polarization. While
4
5 88 the severity of subsequent allergic airway inflammation was affected by AD, abrogation
6
7 89 of AD by topical treatment prevents worsening of subsequent airway inflammation by
8
9 90 counteracting the Th17 pathway¹¹. Granted that both the genetic predisposition and the
10
11 91 atopic progression contribute to the development of atopic march, longitudinal studies are
12
13 92 warranted to explore the impact of immune activation of AD on the development of other
14
15 93 TH2 comorbidities. Meanwhile, the role of epithelial-immune crosstalk in the atopic
16
17 94 march needs to be further elucidated.

18
19 95 To date, extensive research has been conducted on the therapeutic effect of barrier
20
21 96 enhancement using emollients. Compelling evidence indicated that emollients render the
22
23 97 skin less susceptible to irritants and reduce flares of AD (secondary prevention)¹² and
24
25 98 therefore was regarded as a cornerstone of AD therapy¹³. However, evidence was
26
27 99 inconsistent regarding the preventive effect of emollient application^{12 14-17}. The
28
29 100 discordance from these behavioral intervention studies is probably a result of the
30
31 101 difference in when the outcome assessment took place. In the BEEP¹² and
32
33 102 PreventADALL¹⁴ studies, the outcome was measured after a long washout period to
34
35 103 ensure that any mild AD would not be concealed by the on-going application of
36
37 104 emollients, whereas in the small studies^{15 16} the immediate effect was evaluated.
38
39 105 However, the null long-term result should not overshadow the strong efficacy signal
40
41 106 displayed at the immediate outcome assessments. Moreover, as behavior change is a
42
43 107 process that unfolds through a continuum of stages from pre-contemplation,
44
45 108 contemplation, preparation, to action and maintenance, such straightforward behavioral
46
47 109 intervention may not echo with the real-world scenario (the stages of change model),
48
49 110 where behavior changes are achieved through health promotion initiatives, including
50
51 111 health education and health policies^{18 19}. Consequently, the generalizability of these
52
53 112 studies might be affected due to the difference in how intervention was delivered and the
54
55 113 setting under which outcome assessment took place.

56
57 114 In realizing the tremendous burden inflicted by atopic disorders and the critical role of
58
59 115 AD in atopic march, significant therapeutic discoveries have been made over the past
60
116 century. Nonetheless, in AD and other chronic diseases alike, adherence to treatment can
117 be strikingly poor²⁰. The underlying factors were postulated to be the complexity of

1
2
3 118 treatment regimen, corticosteroid phobia, and caregiver burden²⁰. Addressing these
4
5 119 issues calls for consistent efforts in patient education that can hardly be accomplished in a
6
7 120 typical clinical visit. Recent studies have shown that structured patient education can
8
9 121 significantly reduce disease severity in AD patients²¹. Given this, guidelines for AD have
10
11 122 acknowledged education of patients and caregivers as an essential form of secondary
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13 123 prevention to reduce disease flares and improve quality of lives²². However, little is
14
15 124 known about how education performs as a primary prevention approach to reduce AD
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17 125 disease burden.

17 126 Antenatal education is a crucial component of antenatal care. Since its implementation
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19 127 from the nineteenth century, mortality in children has decreased tremendously. In 2005,
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21 128 the World Health Organization called for “realizing the Potential of Antenatal Care”²³,
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23 129 and thence started using it as a platform for primary prevention of malnutrition,
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25 130 HIV/AIDS, sexually transmitted infections, tuberculosis, and prevention of postpartum
26
27 131 and neonatal diseases²⁴⁻²⁶. Its diversified usage has led us to hypothesize that educational
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29 132 intervention delivered through the antenatal education platform may yield informative
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31 133 data for assessing the primary prevention of allergic diseases. Thus, the Preventive
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33 134 Antenatal Education Program on Allergic Diseases (PAEPAD) study focuses on using
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35 135 the antenatal care platform as a more affordable and effective health education approach.
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37 136 In this investigator-blinded, randomized controlled trial, we aim to evaluate the effect of
38
39 137 such intervention vs. standard education on atopic disease outcomes. The primary
40
41 138 objective is to evaluate the preventive effect of PAEPAD on atopic dermatitis and atopic
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43 139 comorbidity incidences. The secondary objectives are: 1) to explore the immune-barrier
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45 140 crosstalk between the immune system, commensal flora and skin barrier function that
46
47 141 may explain the development of atopic march; 2) to identify biomarkers (metabolites,
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49 142 MicroRNAs) that can be used to characterize individuals at high risk of AD and atopic
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51 143 march; 3) to elucidate whether and to what extent the maternal immune milieu influences
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53 144 pediatric AD development through cord blood and breast milk.

50 145 **METHODS AND ANALYSIS**

51 146 **Study design**

53 147 The study is a prospective investigator-blinded randomized controlled trial with two arms
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55 148 (PAEPAD vs. standard antenatal education). All expectant mothers planning to give birth

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3 149 at the Daxing Teaching Hospital of Capital Medical University will be invited to
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5 150 participate. Daxing Teaching Hospital of Capital Medical University is a general hospital
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7 151 located in Daxing district of Beijing, China, with more than 6,000 deliveries annually.
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9 152 Daxing district makes up approximately 6.3% of Beijing geographically. Daxing
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11 153 Teaching Hospital of Capital Medical University makes up approximately 2.7% of
12
13 154 Beijing's annual deliveries. This protocol was drafted (Aug 17th, 2020) before participant
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15 155 recruitment (Oct 30th, 2020).

156 **RECRUITMENT, INCLUSION AND EXCLUSION CRITERIA**

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

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3 **180 Inclusion and exclusion criteria**

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5 181 Run-in phase inclusion criteria: 1. Enlisted in the birth registry of Daxing Teaching
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7 182 Hospital of Capital Medical University and intend to give birth at this location; 2.
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9 183 Women aged ≥ 18 ; 3. Less than 14+6 gestational week when recruited as measured by last
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11 184 menstrual period; 4. Residents of Daxing and intend to remain residing in Daxing for at
12
13 185 least two years postpartum; 5. Written consent form.

14 186 Randomization phase inclusion criteria:

15 187 Pregnant women enlisted in the birth registry of Daxing Teaching Hospital of Capital
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17 188 Medical University and intend to give birth at this location; passes the run-in phase
18
19 189 criteria.

20 190 Withdrawal criteria: 1. Still birth; 2. Abortion (spontaneous or induced); 3. Rare
21
22 191 comorbidities that present after inclusion into the study that may render the participant
23
24 192 unsuitable for participation, including but not limited to malignancies, amniotic fluid
25
26 193 embolism, eclampsia, and major birth abnormalities of the child.

27 194 Exclusion criteria: 1. Planned abortion; 2. Rare comorbidities of the mother that may
28
29 195 cause miscarriage and congenital disabilities as determined by OBs, including but not
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31 196 limited to malignancies, congenital heart diseases, monogenetic diseases; 3. Recurrent
32
33 197 miscarriage; 4. Mental, psychological or intellectual disabilities of either one of the
34
35 198 expecting parents.

36 199 **Treatment allocation**

37
38 200 The study flow is as follows (Fig. 1): Participants will be randomly assigned to one of the
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40 201 two arms (i.e., PAEPAD vs. standard antenatal care). Randomization will be conducted
41
42 202 by an epidemiologist using a computer-generated list with the number of groups being 2
43
44 203 and the distribution ratio of the two groups being 1:1. The list will be generated using
45
46 204 block randomization with block sizes hidden from all investigators. Group allocation will
47
48 205 be placed in sealed opaque envelopes, labeled by numbers only. The envelopes will be
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50 206 opened in consecutive order. Participants will then be informed about their allocated
51
52 207 groups by a research nurse.

53 208 **Blinding**

54 209 This is a researcher-blinded study. Treatment allocation will be performed by an
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56 210 epidemiologist, and the researchers who evaluate the outcome matrices and analyze data

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3 211 will be blinded and work independently from the group of clinicians who will carry out
4 212 the intervention. Data entry will be undertaken by trial administrators blinded to group
5 213 allocation.
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7

8 214 **Intervention**

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10 215 After randomization at GW 30, the participants will be informed on their allocation, and
11 216 members of the research team will send out weekly invitations through messages to
12 217 participants who have not yet completed the intervention. For those who failed to
13 218 complete the intervention prior to admission into the OB department, a pre-recorded
14 219 video will be played during their hospital stay. In the standard care group, patients will
15 220 receive the standard neonatal care session from an experienced obstetrician, which will
16 221 include breastfeeding, newborn screening tests, infant physiology, immunization, solid
17 222 food introduction, belly and eye care (45 min). This session is one of the five mandatory
18 223 sessions with participation of 83.7-91.9% over the past five years (unpublished data). The
19 224 treatment group will receive an educational program designed by a multi-disciplinary
20 225 group of experienced obstetricians and pediatric dermatologists. The program will be
21 226 focused on neonatal care as the control group (45 min) and 1) Skin care of the newborns
22 227 with a practical demonstration on bathing and emollient application (20 min); 2) Sun
23 228 protection (3 min); 3) A brief introduction on commonly used topical agents during
24 229 infancy, including topical corticosteroids, antibiotics, and astringents (5 min); 4) Besides,
25 230 the program will also contain a 5 minutes presentation on atopic dermatitis disease
26 231 burden, its precipitators, managements, disease courses, and the atopic march. Specific
27 232 recommendations are listed in the supplementary table S1. At the neonatal care class, all
28 233 participants will first receive the standard education, which will be held nonconcurrently
29 234 to minimize group contamination. Online courses will be held whenever gathering are
30 235 restricted due to the COVID-19 pandemic. At the end of the sessions, participants of the
31 236 PAEPAD cohort will be asked to participate in the PAEPAD session, which will last for
32 237 less than 40 min. Participants of the standard neonatal care cohort will be asked to leave.
33 238 The intervention will be entirely educational, no cleanser or emollient product will be
34 239 provided or recommended. At follow-up visits, no further education will be implemented.
35 240 Any crossover and non-compliance will be surveyed by a research nurse at the beginning
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241 and end of the antenatal sessions. The research nurses who collect data on compliance
242 and who send out invitations will not be involved in outcome assessment.

243 **Study outcomes and follow-up**

244 Study outcomes

245 All outcome measures are summarized in Table 1. There will be both fixed and disease
246 prompted postnatal follow-up visits during which outcome assessments will take place.
247 Diagnostic criteria for AD will be based on Hanifin&Rajka criteria, which is regarded as
248 the ‘gold standard’ for hospital-based research. The Infants’ Dermatitis Quality of Life
249 Index (IDQoL) is a questionnaire of ten items that has been translated into 21 languages.
250 This questionnaire is validated in infants aged 0–3 years²⁷. Disease severity at disease
251 flares will be measured by SCORing of Atopic Dermatitis (SCORAD) and Eczema Area
252 and Severity Index (EASI) and through the course of the disease by Investigator’s Global
253 Assessment (IGA). SCORAD and EASI are validated instruments for disease severity
254 assessment²⁸. IGA provides the most straightforward assessment of disease severity and
255 will thus be employed to assess AD disease-free days. An IGA of less than two is defined
256 as clearance of disease. An episode of expiratory wheezing will be defined as bronchial
257 obstruction lasting for at least 24 hours preceded by at least a one-week non-wheezing
258 healthy period, as defined by a physician. Recurrent wheezing will be defined as the
259 occurrence of 3 or more episodes of expiratory wheezing diagnosed by a physician in a
260 12-month period²⁹. Rhinitis will be defined as symptoms of sneezing, a runny or blocked
261 nose, or itchy, red and watery eyes after exposure to furred pets or pollen the year before
262 follow-up and/or doctor’s diagnosis of allergic rhinitis³⁰. Sensitization will be defined as
263 allergen-specific IgE ≥ 0.35 kUA/l. Parental knowledge and attitude will be quantified
264 using a questionnaire. The items of the questionnaire were developed following rigorous
265 procedures, including a review of literature, a patient focus group and a panel discussion
266 of experts. This questionnaire is currently under validation (unpublished data).

267 Follow-up

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

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3 272 assessed upon disease onsets and flares. During follow-ups, all cases will be treated
4 273 according to the guidelines^{2 13 31-34} by specialists who are actively engaged in the care of
5 274 pediatric AD patients. Participants are not allowed to participate in other clinical trials
6 275 after inclusion into the study till the end of the last follow-up visit

276 **Covariates**

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

284 **Data collection and management**

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

302 **Biological sample collection**

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

308 **Sample size**

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

316 **Analyses**

317 Definition of population sets

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

325 Statistical analysis

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

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

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3 333 AD episode and time to the first topical corticosteroid exposure, will be calculated using
4 334 the Kaplan-Meier method. The HRs comparing PAEPAD and standard care will be
5 335 estimated using cox regression model. Multiple imputation will be conducted if loss to
6 336 follow-up exceeds 30%. Subgroup analysis will be done for the relative risk of AD,
7 337 asthma, rhinitis and food sensitization stratified by familial history of atopic disorders,
8 338 and AD severity for allergic comorbidities (whenever applies). Regression models with
9 339 interaction terms will be used to test for statistical significance among subgroups.
10 340 For sensitivity analysis that shall be done with the PP population, the analyses above will
11 341 be conducted. For the sensitivity analysis that shall be done with the secondary analysis
12 342 population, both traditional multivariate comparison and propensity score matching will
13 343 be used to better balance the covariates and identify comparable groups. An additional
14 344 sensitivity analysis will be conducted on the population that receives in-person education
15 345 (as opposed to video recorded).

346 **Data monitoring**

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

353 **Patient and public involvement**

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

355 **Ethics and dissemination**

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

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3 364 PI. The results will be disseminated through peer-reviewed journals. Results will also be
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5 365 communicated at scientific conferences.

6
7 366 **DISCUSSION**

8 367 PAEPAD study is a large single-center randomized controlled trial of an antenatal
9
10 368 educational intervention for prevention of atopic dermatitis and the atopic march.
11
12 369 Previous studies on educational interventions have been therapeutic, aiming at reducing
13
14 370 symptoms and improving quality of life. Therapeutic patient education (secondary
15
16 371 prevention) in AD has been proved to be effective with a significant reduction in disease
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18 372 severity²¹. However, little is known about the primary preventive effect of educational
19
20 373 and lifestyle interventions.

21 374 A strength of this study is the large sample size. In a similar study that aims to evaluate
22
23 375 the effect of prenatal education on knowledge and behavioral changes for allergic disease
24
25 376 prevention, the sample size was determined based on the behavioral matrices, resulting in
26
27 377 a total sample size of 120³⁶. As behavioral changes do not necessarily modify disease
28
29 378 outcomes, a larger sample size is warranted to provide adequate power to detect disease
30
31 379 outcome differences. Another strength is that we chose the topics of the PAEPAD
32
33 380 sessions based on both experts' opinion and a previous survey (Supplementary material,
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35 381 table S2), which indicated that new mothers needed help on infant skin care, safe practice
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37 382 of sun protection and building an unbiased understanding on common topical drugs,
38
39 383 especially corticosteroids. We did not discuss about the treatment of atopic diseases
40
41 384 further than emphasizing the importance of following the guideline-oriented instructions
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43 385 of physicians^{2 13 31-34} at this lecture. Recent pilot studies provided strong efficacy signals
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45 386 for the hypothesis that daily emollient use could prevent atopic dermatitis^{15 17}. While the
46
47 387 subsequent large trials yielded null results^{12 14}, it's crucial to realize that the hypotheses in
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49 388 these trials differ significantly. In the pilot studies, emollients were continued until the
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51 389 outcome assessment, whereas in the larger pragmatic trials, a washout period was
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53 390 implemented. In the Barrier Enhancement for Eczema Prevention (BEEP) study, the rates
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55 391 of continued emollient and wash product use extended beyond the intervention period
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57 392 until outcome assessment were as low as four and five percent for the intervention and
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59 393 the control group respectively¹². Thus, the pilot studies assessed the immediate
60 394 preventive effect, whereas the larger studies assessed whether this effect, if there is any,

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3 395 is sustainable. While it's reasonable to hypothesize that the protective effect of emollients
4 396 may not sustain beyond a year after refraining from application, incorporating conceptual
5 397 and behavioral changes into daily skincare routine can be substantially beneficial for
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7 398 short term preventive effects.

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10 399 The current study will explore the preventive effect of an educational intervention, which
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12 400 bears a closer resemblance to the real world scenario through which behavioral changes
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14 401 are achieved. In addition, we plan to longitudinally collect biological specimens to study
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16 402 the crosstalk of lifestyle changes and molecular biology.

17 403 The study has some limitations. First, this study is subject to contamination due to the
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19 404 nature of a non-medication intervention and the single-center design. Consequently, the
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21 405 effect size to be detected will likely be a more conservative estimation of the real
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23 406 preventive effect. Therefore, we plan to collect cross-over data and conduct sensitivity
24
25 407 analyses based on the PP population. Second, although the PAEPAD lectures will be led
26
27 408 by experienced obstetricians and dermatologists, the heterogeneity may nonetheless
28
29 409 constitute potential bias. We aim to reduce the heterogeneity by providing a training
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31 410 session and mock classrooms before the project launching during which lecturers will be
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33 411 evaluated on the organization of the class, clarity, student engagement and consistency of
34
35 412 performance.

36 413 In conclusion, the PAEPAD study will add to our knowledge of the preventive effect of
37
38 414 antenatal education on allergic disease outcomes and identify cellular and molecular
39
40 415 changes that will warrant future studies. We expect that results from the PAEPAD study
41
42 416 will expand our understanding of the primary prevention of allergic disorders.

417 **AUTHORS' CONTRIBUTIONS**

43 418 Lin Ma and Xiuhua Ma are the principal investigators of this trial and conceptualized the
44
45 419 trial. Mutong Zhao, Yuan Liang, Jing Tian, Fengli Song, Ying Wang Lili Ma, Ying
46
47 420 Wang, Wanli Gao are responsible for the execution of the project. Mutong Zhao has
48
49 421 written the protocol manuscript. Chunping Shen, Shan Wang, Lei Jiao, Yang Wang,
50
51 422 Xiaoyan Sun are responsible for data registration. Mutong Zhao and Xiangji Ying will
52
53 423 carry out data analyses once the trial is completed. All authors critically reviewed the
54
55 424 article. All the authors approved the final manuscript.

56 425 Authorship eligibility

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2
3 426 Authorship of the consequent publication of this project will be granted to those who
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5 427 made a significant contribution to the conception, design, implementation, analysis of the
6
7 428 data, or those who drafted the work or reviewed/revised it critically for important
8
9 429 intellectual content.

10 430 Availability of data and materials

11 431 The datasets to be analyzed will be available from the corresponding author upon
12
13 432 reasonable request (requests should be directed to muz880@mail.harvard.edu).

14 433 **FUNDING**

15
16
17 434 This study has received a public grant from the Capital's Funds for Health Improvement
18
19 435 and Research (CFH2020-2-7121). The funders had no role in the design of this study and
20
21 436 will not have any role during its execution, analyses, interpretation of data, or submission
22
23 437 of outcomes.

24 438 **COMPETING INTERESTS**

25 439 None declared.

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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											×
	Gestational diabetes, pre-eclampsia, placenta previa, placental abruption, prelabor rupture of membranes, puerperal infection, postpartum hemorrhage											×
Pathological pregnancy												×
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											×
	Frequency, duration of bathing, bath temperature, cleanser usage, soap usage											×
Bathing practice												×
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

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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		×	×		×					

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578 GW, gestational week

For peer review only

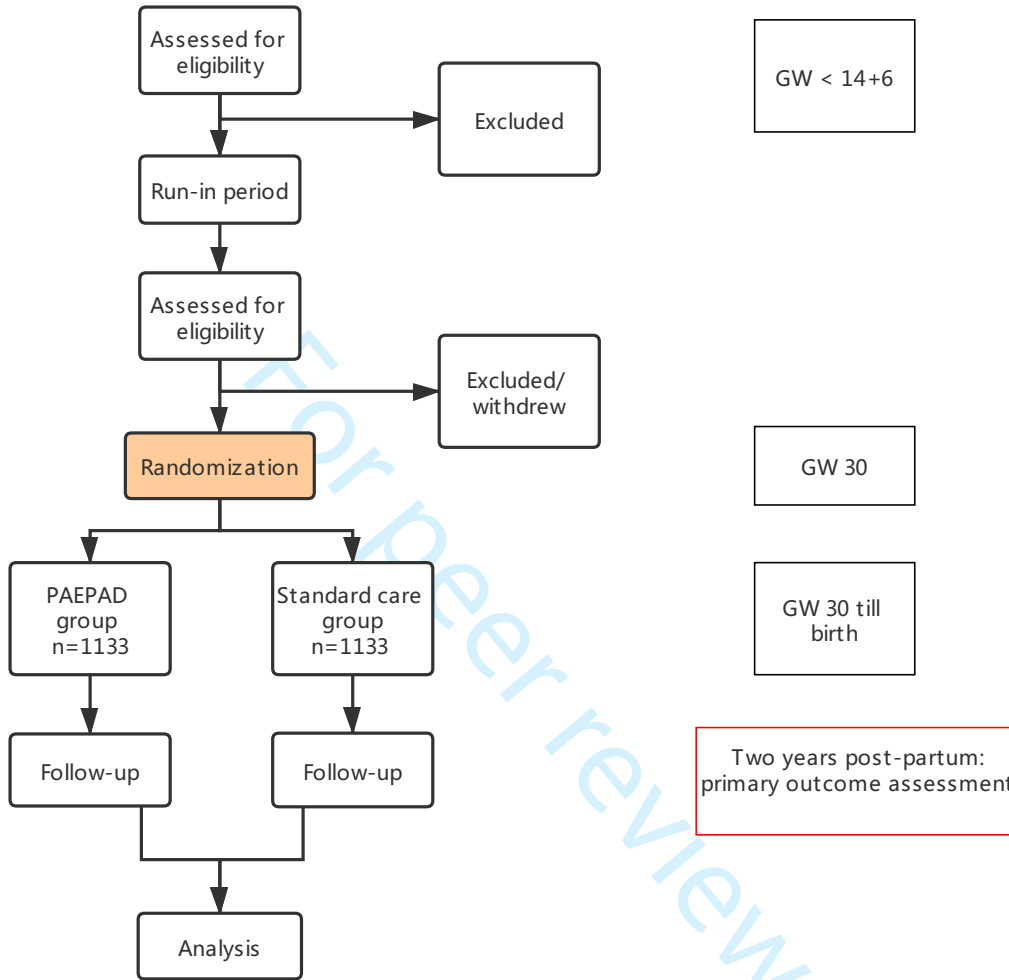
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579 Figure legend

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

581 Diseases) study.

For peer review only



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TABLE S2. SPECIFIC RECOMMENDATIONS ON BABY SKIN CARE PRACTICES6

For peer review only

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 use¹. 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 use².

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

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3 Breast milk will be collected with a breast pump at locations where postnatal follow-ups take
4 place. At follow-up, 30ml of breast milk will be collected with sterilized RNase-free tubes,
5 with the first 500ul disposed. For exosome analysis, 15 ml breast milk will be centrifuged at
6 4°C for 10 minutes at 1500 g to remove cells and the cream layer. The supernatant will then
7 be transferred to new tubes and centrifuged again at 12,000 g at 4°C to remove remaining
8 cells and cream. The supernatant will then be immediately processed or frozen at -80 °C until
9 use³. The remaining 15 ml breast milk sample will be aliquoted and stored at -80 °C directly
10 until use.

17 **Feces**

18 The fecal samples will be self-collected by participants. For 16s RNA sequencing,
19 participants will be provided with a sterile feces collection and preservation kit (ML-001A,
20 Shenzhen Dayun Gene Technology Co., Shenzhen, China). For metabolomics analyses,
21 participants will be provided with a sterile feces collection device with a spatula. Participants
22 will be instructed to collect stool specimens of approximately 10ml within 2 h before each
23 visit and bring them to the visits. Collected feces will then be snap-frozen in liquid nitrogen
24 and stored at -80 °C until use⁴. Meconium of 2ml will be sampled and stored likewise.

31 **Vagina swab**

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

41 **Skin swab**

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

53 **Tape stripping**

54 For each child, skin lipids will be collected by tape stripping from the same body sites as to
55 where skin swabs are sampled from the children. The first layer will be discarded, and the
56 second to fourth layers at the same place will be retained and placed separately in a glass tube
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3 with 5 mL of methanol, then stored at -80 °C until use. The vernix will be sampled from the
4 back of the newborn likewise.

5
6 **Tongue dorsum swab**

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8 Subjects will be instructed not to eat, drink (except water), or brush their tongue during the
9 12 h period before sampling and not to brush their teeth during the 2 h period before
10 12 h period before sampling and not to brush their teeth during the 2 h period before
11 sampling. Specimens will be collected from the central part of the tongue dorsum by swiping
12 for 15 seconds using a sterile swab (4520CA, COPAN Flock Technologies, Brescia, Italy).
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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 sweat 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 eczematous skin.
- 7 Topical corticosteroids used properly will not cause addiction nor resistance.

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 implemented following physician's instructions.
- 5 Topical corticosteroids are the first line therapy for control of inflammation in eczematous children.
- 6 Children with atopic dermatitis are at greater risk for developing other atopic disorders.

Table S2. Responses of a 12-item Knowledge survey

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

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

1				
2	Background and	6a	Description of research question and	3 Line 72,
3	rationale		justification for undertaking the trial, including	supplementary
4			summary of relevant studies (published and	table S2
5			unpublished) examining benefits and harms	
6			for each intervention	
7				
8		6b	Explanation for choice of comparators	5 Line 134
9				
10	Objectives	7	Specific objectives or hypotheses	5 Line 138
11				
12	Trial design	8	Description of trial design including type of trial	5 Line 148
13			(eg, parallel group, crossover, factorial, single	
14			group), allocation ratio, and framework (eg,	
15			superiority, equivalence, noninferiority,	
16			exploratory)	
17				
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19				
20	Methods: Participants, interventions, and outcomes			
21				
22	Study setting	9	Description of study settings (eg, community	6 Line 155
23			clinic, academic hospital) and list of countries	
24			where data will be collected. Reference to	
25			where list of study sites can be obtained	
26				
27	Eligibility criteria	10	Inclusion and exclusion criteria for participants.	6 Line 185
28			If applicable, eligibility criteria for study centres	
29			and individuals who will perform the	
30			interventions (eg, surgeons, psychotherapists)	
31				
32				
33	Interventions	11a	Interventions for each group with sufficient	8 Line 220
34			detail to allow replication, including how and	
35			when they will be administered	
36				
37		11b	Criteria for discontinuing or modifying	Non
38			allocated interventions for a given trial	pharmacological
39			participant (eg, drug dose change in response	intervention.
40			to harms, participant request, or	NA.
41			improving/worsening disease)	
42				
43				
44		11c	Strategies to improve adherence to	8 Line 223-225,
45			intervention protocols, and any procedures for	line 246-248
46			monitoring adherence (eg, drug tablet return,	
47			laboratory tests)	
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50		11d	Relevant concomitant care and interventions	8 Line 244-245
51			that are permitted or prohibited during the trial	
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2	Outcomes	12	Primary, secondary, and other outcomes,	9 Line 264
3			including the specific measurement variable	
4			(eg, systolic blood pressure), analysis metric	
5			(eg, change from baseline, final value, time to	
6			event), method of aggregation (eg, median,	
7			proportion), and time point for each outcome.	
8			Explanation of the clinical relevance of chosen	
9			efficacy and harm outcomes is strongly	
10			recommended	
11				
12				
13	Participant	13	Time schedule of enrolment, interventions	Fig.1
14	timeline		(including any run-ins and washouts),	
15			assessments, and visits for participants. A	
16			schematic diagram is highly recommended	
17			(see Figure)	
18				
19				
20	Sample size	14	Estimated number of participants needed to	11 Line 330
21			achieve study objectives and how it was	
22			determined, including clinical and statistical	
23			assumptions supporting any sample size	
24			calculations	
25				
26				
27	Recruitment	15	Strategies for achieving adequate participant	6 Line 162
28			enrolment to reach target sample size	
29				
30				
31	Methods: Assignment of interventions (for controlled trials)			
32				
33	Allocation:			
34				
35	Sequence	16a	Method of generating the allocation sequence	7 Line 205
36	generation		(eg, computer-generated random numbers),	
37			and list of any factors for stratification. To	
38			reduce predictability of a random sequence,	
39			details of any planned restriction (eg, blocking)	
40			should be provided in a separate document	
41			that is unavailable to those who enrol	
42			participants or assign interventions	
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44				
45	Allocation	16b	Mechanism of implementing the allocation	7 Line 205
46	concealment		sequence (eg, central telephone; sequentially	
47	mechanism		numbered, opaque, sealed envelopes),	
48			describing any steps to conceal the sequence	
49			until interventions are assigned	
50				
51				
52	Implementation	16c	Who will generate the allocation sequence,	7 Line 205, 6
53			who will enrol participants, and who will assign	line 166, 7 line
54			participants to interventions	212
55				
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2	Blinding	17a	Who will be blinded after assignment to	7 Line 214
3	(masking)		interventions (eg, trial participants, care	
4			providers, outcome assessors, data analysts),	
5			and how	
6				
7		17b	If blinded, circumstances under which	Non
8			unblinding is permissible, and procedure for	pharmacological
9			revealing a participant's allocated intervention	intervention.
10			during the trial	NA.
11				
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Methods: Data collection, management, and analysis

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15	Data collection	18a	Plans for assessment and collection of	10 Line 306
16	methods		outcome, baseline, and other trial data,	
17			including any related processes to promote	
18			data quality (eg, duplicate measurements,	
19			training of assessors) and a description of	
20			study instruments (eg, questionnaires,	
21			laboratory tests) along with their reliability and	
22			validity, if known. Reference to where data	
23			collection forms can be found, if not in the	
24			protocol	
25				
26				
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28		18b	Plans to promote participant retention and	9 Line 288
29			complete follow-up, including list of any	
30			outcome data to be collected for participants	
31			who discontinue or deviate from intervention	
32			protocols	
33				
34				
35	Data	19	Plans for data entry, coding, security, and	9 Line 288
36	management		storage, including any related processes to	
37			promote data quality (eg, double data entry;	
38			range checks for data values). Reference to	
39			where details of data management procedures	
40			can be found, if not in the protocol	
41				
42				
43	Statistical	20a	Statistical methods for analysing primary and	11 Line 338
44	methods		secondary outcomes. Reference to where	
45			other details of the statistical analysis plan can	
46			be found, if not in the protocol	
47				
48				
49		20b	Methods for any additional analyses (eg,	11 Line 360
50			subgroup and adjusted analyses)	
51				
52		20c	Definition of analysis population relating to	11 Line 364
53			protocol non-adherence (eg, as randomised	
54			analysis), and any statistical methods to	
55			handle missing data (eg, multiple imputation)	
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Methods: Monitoring

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2	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
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12		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
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18	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.
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24	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
25				
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30	Ethics and dissemination			
31				
32	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	12 Line 380
33				
34				
35				
36	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
37				
38				
39				
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42				
43	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
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48		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	6 Line 170
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52	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
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2	Declaration of	28	Financial and other competing interests for	14 Line 470
3	interests		principal investigators for the overall trial and	
4			each study site	
5				
6	Access to data	29	Statement of who will have access to the final	10 Line 322, 12
7			trial dataset, and disclosure of contractual	Line 386
8			agreements that limit such access for	
9			investigators	
10				
11	Ancillary and	30	Provisions, if any, for ancillary and post-trial	None
12	post-trial care		care, and for compensation to those who	
13			suffer harm from trial participation	
14				
15				
16	Dissemination	31a	Plans for investigators and sponsor to	12 Line 388
17	policy		communicate trial results to participants,	
18			healthcare professionals, the public, and other	
19			relevant groups (eg, via publication, reporting	
20			in results databases, or other data sharing	
21			arrangements), including any publication	
22			restrictions	
23				
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26		31b	Authorship eligibility guidelines and any	14 Line 456
27			intended use of professional writers	
28				
29		31c	Plans, if any, for granting public access to the	Not reported
30			full protocol, participant-level dataset, and	
31			statistical code	
32				
33				
34	Appendices			
35				
36	Informed consent	32	Model consent form and other related	Consent form in
37	materials		documentation given to participants and	Chinese are
38			authorised surrogates	available upon
39				request
40				
41	Biological	33	Plans for collection, laboratory evaluation, and	10 Line 325,
42	specimens		storage of biological specimens for genetic or	supplementary
43			molecular analysis in the current trial and for	material
44			future use in ancillary studies, if applicable	
45				
46				

*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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" 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 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
Contact for scientific queries	Preventive Antenatal Educational Program on Allergic Diseases (PAEPAD) for prevention of atopic dermatitis
Public 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.
Scientific title	
Countries of recruitment	China
Health condition(s) or problem(s) studied	Atopic dermatitis PAEPAD group: multi-disciplinary education of neonatal care
Intervention(s)	Standard care group: standard education of neonatal care 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
Key inclusion and exclusion criteria	

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.

Interventional

Allocation: randomized

Intervention model: parallel assignment

Masking: investigator blind

Study type 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

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.).