Protocol for a Study on the Prevention of Onset of Egg Allergy in Infants with Atopic Dermatitis

PETIT study (Prevention of Egg allergy with Tiny amount InTake)

This file contains the following items:
1) Original protocol, final protocol, summary of changes.
2) Original statistical analysis plan, final statistical analysis plan, summary of changes

The order of items uploaded in this PDF file is as follows:
1. Original protocol
2. Summary of protocol and statistical analysis plan changes
3. Final protocol
4. Original statistical analysis plan
5. Summary of statistical analysis plan changes
6. Final statistical analysis plan changes
Protocol for a Study on the Prevention of Onset of Egg Allergy in Infants with Atopic Dermatitis

Clinical Protocol

Protocol Version 1.0 (Japanese)  (Created February 29, 2012)
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1. Research Title
   Protocol for a study on prevention of onset of egg allergy in infants with atopic dermatitis
   (PETIT study (Prevention of Egg allergy with Tiny amount InTake))

2. Principal Investigator
   Yukihiro Ohya  Chief Physician - Division of Allergy, Department of Medical Specialties, National Center for Child Health and Development

3. Research Organization
   (1) Principal Investigator
       Shall be responsible for managing and supervising the present study.
       Yukihiro Ohya  Chief Physician - Division of Allergy, Department of Medical Specialties, National Center for Child Health and Development

   (2) Sub-investigators

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       Mika Toyomra  Physician - Department of Pediatrics, Tachikawa Sougo Hospital, Kenseikai

   (3) Preparation of Trial powder
       Responsibility for manufacturing and packaging, blinding, and maintaining the quality of trial powder, until it is sent to the research implementing institution.

   Research and Development Department, Kewpie Corporation
   5-13-1, sumiyoshi-cho, futyu-shi, Tokyo     Tel: 042-361-0026,     Fax: 042-361-6271
   Person in charge: Mamoru Kimura

UMIN-CTR: 000008673  Protocol  29-Feb-2012
4. Background and Objectives of the Study

Background

Atopic dermatitis, food allergies, and bronchial asthma are three allergy diseases that are known to have a high prevalence rate in infants, greatly affecting the quality of life (QOL) of those affected. Particularly, food allergies in infants have been increasing in recent years and are turning into a big problem. The onset of food allergies is most common in infants up to one year of age. Most food allergies during this stage are caused by hen’s eggs, cow’s milk, and wheat. Owing to the high daily intake frequency as well as the high nutritional value of these products, a restriction on their intake can greatly affect their QOLs. Moreover, accidental ingestion of an allergenic food can cause serious symptoms such as anaphylaxis, causing the affected infant and their family a great deal of anxiety.

There have been attempts to eliminate allergenic food from the diets of children as well as those of pregnant and nursing women in order to prevent the onset of food allergy. However, such attempts have been consistently reported to have failed. In its publication in the year 2000, the American Academy of Pediatrics (AAP) advocated for delayed introduction of baby foods known to be highly allergenic, but in 2008, it reversed these guidelines. Instead, recently there have been a few reports showing that an early introduction to highly allergenic foods lowers the frequency of food allergies\(^1\)\(^2\).

On the other hand, a prospective cohort study implemented at our center has revealed a strong association between food allergy and atopic dermatitis. Children who contracted eczema within first 6 months of age showed an odds ratio of 10-20 for contracting a food allergy by the age of 1 ~ 3 compared to children who did not contract eczema\(^3\).

Currently, a new idea known as the dual-allergen-exposure hypothesis is gaining attention as an explanation for this phenomenon\(^4\)\(^5\). As per this hypothesis, patients with atopic dermatitis have a disrupted skin barrier that allows exposure to food proteins, which results in the development or strengthening of allergy antigen sensitization. On the other hand, the ingestion of food proteins induces an immunological tolerance. This hypothesis suggests that immunological tolerance by early introduction to antigenic food proteins through baby foods, or inducing preventing percutaneous sensitization by maintaining the skin in a healthy state during infancy may have a preventive effect on the onset of food allergies.

Objective

In the present study, we shall focus on hen’s egg, described in a preceding retrospective study as the most common food that leads to onset of food allergy during infancy. The study targets are 4~5-month-old infants with atopic dermatitis, as they are considered to be a high-risk group for developing food allergies. After daily oral administration of heated whole egg powders to a large number of infants for 6 months (from the time they are 6 months old and start baby food until they turn 1), we will implement an oral oral food challenge once they have completed 12 months of age to investigate whether or not they have developed an egg allergy. In this way, we will
investigate the efficacy of introducing egg from an early stage of baby food as a preventive therapy against the onset of immediate-type egg allergy. Also together, patient’s skin condition is continuously observed to evaluate the correlation between the strength and extent of dermatitis and the incidence of egg allergy. Then the preventive effect of onset of egg allergy by the treatment of atopic dermatitis is evaluated.

5. Subjects of Study

Patients with atopic dermatitis who meet all of the following inclusion criteria and do not fall under any of the exclusion criteria.

Inclusion Criteria

① Patients who are 4 or 5 months old at the time of registration.
② Patients diagnosed with atopic dermatitis according to the Hanifin and Rajka criteria.
③ Patients who will be able to visit the out-patient units of the hospitals for following up every one or two months, until receiving the oral food challenge test at 12 months of age.
④ Patients who will be able to undertaken the oral food challenge test at 12 months of age.
⑤ Patients for whom egg intake can be completely eliminated from daily life during intervention, except when given as a trial powder and for the oral food challenge.
⑥ Patients for whom informed written consent has been obtained from the guardian.

Exclusion Criteria

① Patients born before 37 weeks of gestation.
② Patients who have already been introduced to hen’s eggs.
③ Patients who have a history of immediate-type egg allergy.
④ Patients suspected to have a particular food allergy other than immediate-type response.
⑤ Patients judged by the attending physician as having a serious coexisting disease that could cause hindrance in the implementation of this study.
⑥ Patients judged to be medically unfit for participation in this study by the attending physician.
6. Target Sample Size and Basis for Establishment Thereof

Target Sample Size

- Egg group: 100 infants
- Placebo group: 100 infants
- Total: 200 infants

Basis for Establishment

According to studies\(^1\)\(^,\)\(^6\)\(^,\)\(^7\) on the incidence rate of immediate-type allergy in infants with atopic dermatitis reported so far, a nation-wide prevalence study\(^8\) on atopic dermatitis and immediate-type food allergies, as well as the data obtained from a birth cohort study (unpublished) being implemented at this center, the incidence rate of immediate-type hen’s egg allergy in 1 year old infants with atopic dermatitis is estimated to be 7% when intervened by intake of egg, and 20% in case of placebo intake. In this study, the sample size needed to detect the difference with 80% power using a two-sided test (5%) is 92 subjects in each group. There will be 100 subjects in each group, presupposing a dropout rate of about 10%.

7. Study Duration

Scheduled to start after approval from the ethics committee and end by March 31, 2015.

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**Hanifin and Rajka Diagnostic Criteria for Atopic Dermatitis**

- **C** Must have 3 or more basic features
  - **Pruritus**
  - Typical morphology and distribution:
    - Flexural lichenification or linearity in adults
    - Facial and extensor involvement in infants and children
  - Chronic or chronically-relapsing dermatitis
  - Personal or family history of atopy (asthma, allergic rhinitis, atopic dermatitis)

- Prus 3 or more minor features:
  - **Xerosis**
  - Ichthyosis/palmar hyperlinearity/keratosis pilaris
  - Immediate (type 1) skin test reactivity
  - Elevated serum IgE
  - Early age of onset
  - Tendency toward cutaneous infections (esp. Staph. Aureus and Herpes simplex)/impaired cell-mediated immunity
  - Tendency toward non-specific hand or foot dermatitis
  - Nipple eczema
  - Cheilitis
  - Recurrent conjunctivitis
  - Dennie-Morgan infraorbital fold
  - Keratocanthoma
  - Anterior subcapsular cataracts
  - Orbital darkening
  - Facial pallor/facial erythema
  - Pityriasis alba
  - Anterior neck folds
  - Itch when seating
  - Intolerance to wool and lipid solvents
  - Perifollicular accentuation
  - Food intolerance
  - Couse influenced by environmental/emotional factors
  - White dermographism/delayed blanch

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8. Study Methodology

(1) Type of trial

A double-blind, parallel group, stratified, randomized controlled trial

Basis for Establishment

In the present study, a hen’s egg daily intake group and a placebo group will be formed and compared in order to investigate the safety and efficacy of food allergy preventive therapy, in which egg will be given in early phase of baby food.

We decided to conduct a double-blind study to eliminate any bias on the part of the subjects or the researchers during the evaluation of safety and efficacy of hen’s egg intake. Moreover, placebo control is necessary as it is impossible to accurately estimate the incidence rate of immediate-type allergies in infants.

The burden of daily intake of trial powder for a period of 6 months on the subject and their family is considered to be permissible. Moreover, currently there is a tendency to introduce eggs in baby food once the infant is 7-8 months old, with it being common to prescribe long-term elimination of eggs as a part of treatment, particularly for infants with atopic dermatitis. In the present study, eggs will be completely eliminated for the placebo group until the subjects complete 12 months of age, which is considered to be within the permissible range.

(2) Outline of the Trial

From among the 4-5 month old infants diagnosed with atopic dermatitis according to the Hanifin and Rajka criteria, those for whom consent for participation in the study is obtained from the guardians will be registered. Subjects will be randomly assigned to either the "Egg group" who will ingest hen’s eggs, or the "Placebo group" who will ingest placebo. Once the subject turns 6 months of age, 300 mg/day of trial powder composed of 50 mg of heated whole egg powder, 100 mg of pumpkin powder, and 150 mg of glucose powder will be started for the Egg group, whereas trial powder composed of pumpkin powder (150 mg/day) and glucose powder (150 mg/day) will be started for the placebo group. For the sake of safety, both groups will be given their first intake at the hospital as outpatients. The same amount of intake per day will be continued until they are 9 months old. Once the subject turns 9 months of age, the intake amount will be increased to 500 mg/day of trial powder composed of
heated whole egg powder 250 mg and glucose powder 250 mg for the Egg group, and trial powder composed of pumpkin powder 250 mg and glucose powder 250 mg for the Placebo group. For the sake of safety, both groups will be given the first increased amount of trial powder as outpatients at the hospital. Hereafter, the same amount of intake will be continued until they are 12 months old. Once the subjects turn 12 months of age, an oral food challenge using boiled egg white will be implemented as in-patients to investigate whether or not they have developed an egg allergy.

In the meantime, the participants will be treated atopic dermatitis with proactive therapy or reactive therapy based on caregiver’s preference. Participants whose caregiver had no preference of proactive therapy or reactive therapy are allocated to either therapy to equalize the participant number of each therapy group.

**Basis for Establishment**

The initial intake quantity of heated whole egg powder was established as 50 mg (equivalent to 0.2 g of whole egg boiled in hot water for 15 minutes) after referring to previously reported allergen thresholds for hen’s egg, and symptom provocation thresholds established in food challenge tests conducted by previous studies using egg powder ("Study on oral desensitization treatment including rush induction using heated egg powder" and "Study on oral desensitization treatment with slow induction using heated egg powder") and after considering its quantifiable feasibility as food.

Pumpkin powder was selected as the placebo powder as it has extremely low allergenicity, is widely used as initial-stage baby food, and is similar to boiled egg powder in terms of color and texture. Glucose is also added to improve the liquidity of the powder.

(3) Registration and Allocation

**Registration Process**

After obtaining consent for participation in the study, the physician-in-charge will confirm that the subjects meet all the subject inclusion criteria and do not fall under the exclusion criteria, after which physicians will enter the anonymization numbers, registration date, date of birth, and gender in the case registration table, and will contact the data center at the address given below. At the data center, the subjects will be stratified on the basis of gender and registration institution, and equal number of subjects will be allocated to the Egg group and the Placebo group.

[Contact address for case registration]

Clinical Research Center, National Center for Child Health and Development (Hereinafter referred to as the "Data Center")

Person in charge: Tetsuya Takimoto, Division Chief, Department of Clinical Research Promotion
Eligibility Verification

The Data Center will verify the eligibility of the target group.

1) Subjects shall not be registered if the registration table is not filled in properly.
2) Once registered, the subjects shall not be deleted.
3) In case of double registration, the information registered the first time shall be used.

Participant Assignment

The participants are randomly allocated to the egg group or the placebo group (1:1) by the data center.

(4) Atopic Dermatitis Treatment Method

The way of proactive therapy for atopic dermatitis is to prevent skin flare proactively by using intermittent topical corticosteroids during the maintenance phase, and that of reactive therapy is to apply topical corticosteroids only when eczema is exacerbated.

① proactive therapy group
In the first stage of the therapy, participants and their caregivers are given a lecture to educate skin care treatment and application of ointments. After their eczema is cleared by application of topical corticosteroids, the therapy moves into the maintenance phase to prevent flare by using emollient and intermittent topical steroids which are tapered gradually based on the physician’s advice. They can visit outpatient units to see the doctors in addition to their scheduled visits.

② reactive therapy group
In the first stage of the therapy participants are and their caregivers are given a lecture to educate skin care treatment and application of ointments. They are advised to use topical steroids when their skin condition is not controlled. They can visit outpatient units to see doctors in addition to their scheduled visits.
The choice of proactive therapy or reactive therapy may not be changed through the trial, so long as caregivers do not want to.
When the skin condition got worse, physicians apply the best treatment at that point and continue the trial.

Basis for Establishment

According to the dual-allergen-exposure hypothesis mentioned earlier, stimulation with dietary protein via skin strengthens sensitization to food allergens, but the degree of sensitization may vary depending upon the skin condition. Whether influence of proactive therapy for eczema on percutaneous sensitization of food allergens is different from that of reactive therapy has remained to be elucidated. We respect caregiver’s preference of either eczema therapy and random allocation of each therapy is not applied. Therefore difference of the effect of each eczema therapy on percutaneous sensitization is analyzed by devidint into two subclasses without randomization.
(5) Amount, Period, and Method of Trial powder Intake for Each Group

**Intake Amount and Intake Period**

1. **Egg group**
   - [From 6 months of age until 8 months of age]
     - Mixture of 50 mg of heated whole egg powder, 100 mg of pumpkin powder, and 150 mg of glucose powder
   - [From 9 months of age until 11 months of age]
     - Mixture of 250 mg of heated whole egg powder and 250 mg of glucose powder
   - Storage method: Cryopreserved

2. **Placebo group**
   - [From 6 months of age until 8 months of age]
     - Mixture of 150 mg of pumpkin powder and 150 mg of glucose powder
   - [From 9 months of age until 11 months of age]
     - Mixture of 250 mg of pumpkin powder and 250 mg of glucose powder
   - Storage method: Cryopreserved

For both the groups, the trial powder packets to be consumed each day will be provided beforehand to the guardians, and feed of 1 packet per day will be started once the infant is 6 months old ± 2 weeks. The daily intake will be continued for a period of 6 months, that is, when the infant is between 6 ~ 11 months of age. During this period, the quantity of trial powder will be increased once the infant is 9 months old ± 2 weeks. For the sake of safety, the first feed at 6 months and subsequent quantity increase at 9 months will be given at the hospital as an outpatient.

**Intake Method**

The trial powder intake for both the groups will be 1 packet per day. There is no particular feeding method and the trial powder can be given separately or by mixing with other food like porridge; however, it must not be heated at over 100°C, for example, cooked in the oven etc.

**Discontinuation of Intake when Unwell**

The trial powder will not be given when the infant is unwell, for example with gastroenteritis, fever, etc. If the intake of test sample has been discontinued for more than 3 days due to an illness, when restarting, the trial powder quantity will be cut down to half the prescribed amount for the initial 2 days.

**Basis for Establishment**

The trial powder must not be cooked at over 100°C as the hen’s egg protein undergoes denaturation if processed at high temperatures, such as in the oven, thereby weakening its properties as a food allergen, and may therefore hinder the development of the immunological tolerance expected through the introduction of continued intake of the trial powder.

Even normally well-tolerated food may induce allergic symptoms when a child is physically unwell with gastroenteritis, etc. Therefore, if the intake of trial powder is temporarily discontinued owing to an illness, it must be cautiously restarted by reducing the intake quantity to half.
(6) Trial powder

Overview of trial powder is given below.

**Heated whole egg powder**

Preparation method: Heated (95°C for 15 minutes), pulverized, heat sterilized, spray-dried
Additives: None
Egg production area: Japan
Manufacturer: Kewpie Corporation
Powder packaging: Glip Co., Ltd.
Warranty period from date of manufacture: 6 months when stored under -20°C in hermetically sealed individual packets
Microbiological standards: General viable bacterial count 5000/g or less, fungi/yeast 300/g or less
E. Coli/Staphylococcus aureus/Salmonella negative
Contamination: The trial powder will be checked for soybean/milk/wheat contamination using FASTKIT Elisa Ver. II (antigen measurement kit manufactured by Nipponham).
The Kewpie Corporation which handles the manufacturing and individual packaging offers guarantee for the specifications mentioned above.

**Pumpkin Powder** (Vegetable fine powder – KABOTYA®)

Preparation method: Heat and steam sterilized, desiccated, jet-stream pulverized
Additives: None
Pumpkin production area: Japan
Manufacturer: Mikasa Sangyo Co., Ltd.
Guarantee period from date of manufacture: 24 months when stored under normal temperature in a cool dark place
Contamination: As per the Food Sanitation Law, 25 allergic substances cannot be manufactured by pulverization inside the same factory.
Mikasa Sangyo that handles the manufacturing offers guarantee for the specifications mentioned above.

**Glucose Powder** (Showa anhydrosugar powder)

Additives: None
Production area: Japan
Manufacturer: Showa Co., Ltd.
Quality guarantee period from date of manufacture: One year from manufacture when stored at normal temperature
Contamination: As per the Food Sanitation Law, 25 allergic substances cannot be manufactured by pulverization inside the same factory.
(7) Restrictions

Intake of eggs and food products containing eggs is prohibited when the infant is between 6-12 months of age and is being fed the trial powder. The nursing mother is not prohibited from consuming eggs and egg-containing foodstuff even when the infant is being breast-fed.

[Basis for Establishment] Intake is prohibited as intake of egg-containing foodstuffs by the subject during the trial period may influence the efficacy evaluation of the study. Even if the nursing mother consumes egg-containing foodstuffs, only a minuscule amount of egg protein is secreted in the breast milk, which may have a much smaller effect on efficacy evaluations of the study therapy. Additionally, complete elimination of egg-containing foodstuff from the mother's diet may greatly affect her life, and therefore has not been restricted.

(8) Oral food challenge

Oral food challenge Implementation Period

The oral food challenge will be implemented between 2 weeks before the infant's 1st birthday and 4 weeks after it.

Overview of Oral food challenge

The oral food challenge will be implemented as an open method, in which the contents of the food to be ingested will be known to the tester and the subjects. The infants will be given 1 g, 2 g, and 5 g of boiled egg white, which is boiled for over 20 min, every 30 minutes (total 8 g). The results will be judged as "positive" if the ingestion induces any symptoms such as, hives, nasal discharge, cough, vomiting, abdominal pain, etc. Further intake will be stopped if symptoms appear while the infant is being fed. The results will be judged as "negative" if a total of 8 g of intake does not induce any particular symptom.

The oral food challenge will be implemented at NCCHD or Tachikawa Sougo Hospital. The same procedure will be used at both hospitals. The participants decide which hospital they will take the challenge test in.

[Basis for Establishment]

A double-blind, placebo-controlled oral food challenges the gold standard for diagnosis of immediate-type food allergies. However, the one-year-old subjects of the present study are unlikely to have psychogenic reactions. Therefore, an open method, which can be implemented easily and has less loads on the subjects, is expected to give a highly credible assessment. Therefore, an open method, which has less loads on the subjects, is expected to give a highly credible assessment.

9. Endpoints

(1) Primary Endpoint

Negative rate of diagnosis from oral food challenge implemented on 12 months olds (open method)

[Basis for Establishment]

A oral food challenge (double-blind trial) is the gold standard when it comes to diagnosis of immediate-type food allergy. As mentioned earlier, since the subjects being evaluated are 1-year old infants with negligible psychogenic reactions, an open oral food challenge is considered to be adequately reliable. Additionally, an open method reduces the loads on the subjects by half as compared to conducting the test as a double blind method.
(2) Secondary Endpoints

① Serum hen’s egg-specific IgE at the start of the study, after 3 months, at the end.

② Serum hen’s egg and other food* specific IgG1, IgG4, and IgA (a novel allergen microarray system, which is equipped with a DLC-coated chip, as previously published (Suzuki K, Anal Chim Acta 2011; 706:321-7.)) at the start of the study, after 3 months, at the end.

* other food: milk, wheat, soy, peanut, buckwheat, rice, beef, chicken, house dust, mite, dog, cat.

③ Flow cytometric analysis of blood cell at the start of the study and at the end.

④ Serum TARC (thymus and activation-regulated chemokine) at the start of the study, after 3 months, at the end.

⑤ Salivary IgA, IgG1, and IgG4 measurements (DLC-coated chip) at the start of the study, after 3 months, at the end.

[Basis for Establishment]

Antigen-specific IgE is an index of sensitization to dietary proteins that cause of food allergies.

IgG1, IgG4, and IgA are important factors of immunoreaction in the onset of food allergies. A blood examination will be performed in order to assess immunological change during the intervention and also to evaluate sensitization to other foods. Association with immunological tolerance will be investigated by comparing specific salivary IgA, IgG1, and IgG4 antibodies with serum IgE.

10. Observation Items

(1) Background of participants

The following items will be investigated before the registration process.

① Gender, date of birth, gestational age, weight, patient's initials, clinical records number

② Mode of nutrition (exclusive breast feeding, mixed feeding, or bottle feeding)

③ Complication or history of food allergy

④ Regular medication

⑤ Other coexisting disease

⑥ Family history

(2) Status of intake of trial powder as well as compliance with antigen eliminated diet

At every visit as an outpatient, the logs maintained by the guardian will be checked.

(3) Confirmation of adverse events

At every visit as an outpatient, the logs maintained by the guardian are checked.

(4) Symptoms of atopic dermatitis

The guardians will evaluate and record the symptoms related to skin findings observed by them once a week using POEM (Patient-Oriented Eczema Measure) between 6 months ~ 12 months of age. Additionally, the attending physician will evaluate and record the SCORAD (severity scoring of atopic dermatitis) at the start of the
trial (4 to 5 months of age) and when implementing the oral food challenge at 12 months of age.

(5) Observation and Examination Schedule
The above observations and examinations will be implemented based on the following examination schedule table.

<table>
<thead>
<tr>
<th>Implementation items / implementation period</th>
<th>First visit</th>
<th>Enroll (when 4~5 months old)</th>
<th>6 months old$^{1)}$</th>
<th>Regular checkups every 1~2 months</th>
<th>9 months old$^{2)}$</th>
<th>Regular checkups every 1~2 months</th>
<th>1 year old$^{3)}$</th>
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<tbody>
<tr>
<td>Consent acquisition</td>
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<td>Investigation of subjects’ background</td>
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<tr>
<td>Dermatitis treatment</td>
<td>Start</td>
<td></td>
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<td>Complete (continued if required)</td>
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<td>Skin findings (SCORAD) assessment</td>
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<tr>
<td>Skin findings (POEM) assessment</td>
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<td>Test food intake as outpatient</td>
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<td>Test food intake</td>
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<td>Test food intake status check</td>
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<tr>
<td>Food challenge test (egg albumin intake)</td>
<td>○</td>
<td></td>
<td></td>
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<td></td>
<td>○</td>
<td></td>
</tr>
<tr>
<td>Questionnaire</td>
<td></td>
<td></td>
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<td>○</td>
<td></td>
</tr>
</tbody>
</table>

1) 2) Age in months ± 2 weeks
3) Age in months ± 3 weeks

11. Withdrawal Criteria
Any subject falling under any of the following withdrawal criteria will be withdrawn from the study

① Guardian of the subject has refused participation in the trial or has revoked the consent.
② The trial powder causes symptoms of immediate-type allergy during the trial period. Symptoms of immediate-type allergy are adverse events occurring within 120 minutes of consuming the trial powder, manifesting in the form of skin symptoms, respiratory symptoms, mucous membrane symptoms, gastrointestinal symptoms, or abdominal symptoms. Additionally, if continuation in the trial becomes difficult due to other adverse event.
③ If continuation of the intervention becomes difficult due to aggravation of complications.
④ If judged as not fulfilling the eligibility criteria after the registration.
⑤ The subject stops to visit during the trial and becomes untraceable.
⑥ The entire trial has been discontinued.
⑦ The doctor finds it appropriate to discontinue the trial for some other reasons.

12. Handling of an adverse event

① Dealing with the subjects in case of an adverse event

The study representative or the sub-investigators will immediately take appropriate medical measures upon observing an adverse event.

② Reporting of serious adverse events

A serious adverse event is an unfavorable event fitting any of the following definitions provided in points ① ~ ⑤.

① Death
② Life-threatening event
③ Event requiring hospitalization or extension of hospitalization period
④ Event leading to disability or impairment
⑤ Later-generation congenital disease or abnormality

The adverse events that must be reported include; all serious adverse events that occur during the trial participation, serious adverse events suspected to be associated with the trial powder after completion (discontinuation) of study therapy, as well as other adverse events for which medical reporting is judged to be necessary by the study representative. However, this does not include hospitalization for the purpose of the oral food challenge scheduled for the trial.

Upon observing the occurrence of the above-mentioned adverse events, the study representative will promptly send a written report to the center head and the ethics committee through the ethics committee office, to seek their review and judgment regarding advisability of continuation with the trial.

13. Statistical Analysis

(1) Analysis Set

FAS (Full Analysis Set)

FAS consists of all randomized subjects who take at least one trial powder

PPS (Per Protocol Set)

PPS consists of all randomized subjects who take trial powder and fulfill the following criteria.

① Fulfill all the inclusion criteria and do not meet the exclusion criteria.
② Take trial powder for at least 130 days.
③ Successfully eliminated hen’s-egg containing foodstuff from the diet, except for trial powder, with accidental ingestion during trial limited to a maximum of 3 times.

The primary analysis set for efficacy and safety analyses is FAS and secondary analysis set is PPS. Handling of FAS and PPS is determined for each endpoint.
(2) Definition of case categorization

Eligible cases: Cases that fulfill all the inclusion criteria and do not meet the exclusion criteria

Withdrawal cases: Cases that are withdrawn from the trial due to the criteria mentioned in Section 11

(3) Data Handling

Handling of withdrawal case data

① As to FAS, results of participant’s blood examinations and an oral food challenge test are used as outcomes even if they were carried out before the end of the trial. If those data do not exist, outcome data of the case are handled as missing values.

② As to PPS, results of participant’s blood examinations and an oral food challenge test are used as outcomes even if they were carried out before the end of the trial. If those data do not exist, outcome data of the case are handled as missing values.

③ Data fixed before the release of blinded assignment should be used as outcomes to be analyzed.

Supplementation of missing values of oral food challenge tests. Handling after violation matters

If the data of withdrawal cases are recognized as "missing values", their results are handled as positive in the oral food challenge tests. The results of primary endpoint (oral food challenge) who had violation matters are treated as follows in FAS and PPS.

① Cases who take the trial powder for less than 130 days: the result of the oral food challenge test is used as primary endpoint in FAS.

② Cases who accidentally ingested egg containing foods other than the trial powder in four or more times: the actual measurement of the oral food challenge is used as primary endpoint in FAS.

③ Cases whose diary was lost or blank: the result of the oral food challenge test is used as primary endpoint in FAS and PPS.

Missing values caused by withdraw and positive results of endpoint caused by violation matter are recorded in the analytical procedure, and those are excluded in the data summary.

The other

Handling of cases and data not described in this protocol such as data fixation whether they should be included or excluded are determined after discussion among statistician and trial investigators. The key open is performed after data fixation.

(4) Analysis of Efficacy

Interim Analyses

One interim analysis is planned in this study. One year after the beginning of the study or when 100 subjects completed this study. Difference between the two groups as to primary endpoint is checked to recalculate the sample size and assess safety of the trial.
Descriptive statistics

Protocol violations
In each group, the number of withdrawals are summarized. This description is done separately for FAS and PPS.

Status of intake of trial powder
The number of days of the trial powder consumed is summarized.

Summary of data
For each groups, serum immunological test readings, POEM values is summarized.
For each groups, diagnosis of oral food challenge is summarized.

Summary of background
For each groups, gender, nutrition during infancy, history of allergic diseases of both parents is summarized.
For each groups, serum TARC. is summarized.

Analysis of Efficacy Endpoints
① Endpoint is Negative rate of diagnosis from oral food challenge implemented on 12 months olds (open method)
② Participants are evaluated about endpoint at the end of the trial.
③ Analysis of the Primary Endpoint
For the primary comparison, oral food challenge results (positive/negative) of each intervention group at 12 months of age are compared using the Fisher's exact test. FAS is used as the primary analysis set. Secondary analysis is made using PPS. Level of significance is set at 0.05. The 95% confidence interval of the group difference is calculated using binomial distribution.
As secondary evaluation, the primary endpoint is analyzed adjusted for severity of atopic dermatitis evaluated using SCORAD, feeding by breast milk, and history of allergic diseases of both parents are conducted using the logistic regression model.

Analyses of Secondary Endpoints
Difference of oral food challenge results (positive/negative) between proactive therapy and reactive therapy is detected by using the Fisher's exact test. Level of significance is set at 0.05. The 95% confidence interval of the group difference is calculated using binomial distribution.
Analyses adjusted for severity of atopic dermatitis evaluated using SCORAD, feeding by breast milk, and history of allergic diseases of both parents are conducted by using the logistic regression model.
The other endpoints to evaluate the alteration between before and after the trial are as follows.
① Serum hen’s egg-specific IgE (egg white and ovomucoid)
② Serum antigen specific IgG1, IgG4, and IgA
③ Serum CD4+ CD25+ Treg, inflammatory Th2 cell and inflammatory cytokine
④ Salivary IgA, IgG1, and IgG4 concentrations at the start of the study and after 6 months from the trial
end.

⑤ SCORAD score and POEM score

Wilcoxon signed rank test is applied for comparing changes within the group, and Wilcoxon rank sum test is used for comparing the difference between the groups as to methods of therapy for atopic dermatitis and trial powders.

**Analysis of Safety Endpoints**

Adverse events is classified into serious and not serious, the name of adverse event, number of cases in which they appeared, and frequency of occurrence is calculated. Name of adverse reaction, number of cases in which they appeared, and frequency of occurrence is calculated.

14. Expected Outcome and Significance of the Study

At present, ways of preventing the development of food allergy through baby food are not known, and the introduction of highly allergenic foods in infants is delayed with no particular basis evidence. If it can be ascertained that early introduction of such foods decreases the development of immediate-type food allergy, it will be proven that the existing intake theory rather increases the onset of food allergies, and early intake may be recommended. This will constitute a new finding and may change the nutrient intake methods for all infants, making a huge contribution to the public and society.

15. Advantages and Disadvantages of Participation in the Study

**Advantages of Participation**

① Egg intake can be started safely as egg will be introduced for the first time under the supervision of a physician.
② If food allergy appears, it can be promptly treated by a medical specialist.
③ The participant will undergo regular medical checkups and receive appropriate treatment for atopic dermatitis by a medical specialist.

**Disadvantages of Participation**

① The participant must consume the prescribed amount of trial powder everyday at home.
② The participant cannot be given eggs or egg-containing foodstuff during the trial period.
③ Progress of the intervention must be recorded and the participant must undergo regular medical checkups once in every 1~2 months.
④ The participant must take the oral food challenge.
⑤ The participant must undergo 3 blood collections for the tests.
⑥ If the infant is already allergic to eggs at the time of enrollment in the study, there is a possibility of appearance of strong allergy symptoms, such as hives or anaphylaxis, upon consuming the trial powder.

16. Right to Participate in and Withdraw from the Study
Since the targets of the present study are infants, it is not possible to obtain their consent. Therefore, the principal investigator or the sub-investigators will obtain voluntary written consent from participants’ parents or their guardians, after adequately explaining the briefing paper containing the following explanatory items. The consent form will include the title of items explained, the full name of the subject, relationship of the legal representative for the subject, signature of the legal representative, and date of filling the form. The briefing paper will be handed over to the subject and his/her legal representative, while the consent forms will be in the custody of the study representative until completion of the present study or until 5 years have elapsed after withdrawal.

Consent can be revoked at any point of time. However, it will be impossible to remove specific individual data if the study results have been published after immobilization/analysis.

[Explanatory items]
① Objective of the study
② Study methodology
③ Study implementation period and planned duration of participation
④ The number of participants scheduled to participate in the study
⑤ Expected clinical benefits as well as disadvantages
⑥ The compensation and treatment available to the participant in the event of a study-related health hazard
⑦ That the subject’s participation in the trial is voluntary and they can revoke the consent whenever they choose. That the participant's refusal of or withdrawal from participation in the study does not cause any disadvantage to the participant.
⑧ That the guardian of the participant will be informed by the physician in a timely manner if information becomes available that may be relevant to the participant's willingness to continue participation in the study.
⑨ Withdrawal criteria for participation in the study
⑩ That the personal information of the participant will be protected while the study is being carried out and when the results are published.
⑪ The participant's responsibilities.
⑫ Full name, official title and contact address of the study representative, and contact address of the medical institution providing the consulting service.

17. Cost Burden

Cost burden of the trial
The participants will bear the cost of the hospital stay for the food challenge, charges for some of the blood tests, and charges for outpatient visits every 1~2 months, which are all covered under health insurance. For the work load on the subjects for participating in the study in the form of daily intake of trial powder and maintaining weekly records of skin condition, a remuneration of JPY 500 QUO Card per subject will be granted.

The remaining tests (IgG1, IgG4, and IgA) not covered under health insurance will be paid for through research funds and thus will not be borne by the subjects. The trial powder will be purchased using the research expenses
and will be distributed to the subjects free of charge.

The food will be provided by the Kewpie Corporation, which our center has no profit sharing in. There is no load related to analyses on the participants other than tests conducted for medical examination and treatment.

**Funding sources and financial relations**

The present study will be implemented using MHLW research grants and grants from the NCCHD for medical research and development in all fairness, with the funding parties having no relationship with any of the researchers involved in the study.

18. **Protection of Personal Information and Handling of Research Results**

The registered patients will be identified or referred to using the registration numbers issued at the time of registration.

At the time of publishing the research results, the privacy of the individuals will be protected by anonymizing the research data. The research results are regarded as highly valuable as the basis for preventing onset of food allergies in infants, and will be shared with the public in the form of reports at domestic academic conferences and publications in domestic and international journals. The research results will be the intellectual property of the Division of Allergy, National Center for Child Health and Development.

19. **Compensation for Health Hazards and Taking out Insurance**

If any sort of damage to a participant's health results from the present study, the study representative and sub-investigators will promptly provide diagnosis and treatment within the scope of healthcare services provided by health insurance. The study representative and sub-investigators will separately take out medical professional liability insurance.

20. **Regulations to be Complied with**

The present study will be implemented in compliance with the final protocol, which has been created in accordance with the Helsinki Declaration and ethical principles related to clinical research.

21. **Handling of Samples after Completion of the Study**

Samples such as left-over serum, etc., will be kept anonymous and stored under refrigeration for a period of one year after completion of the study (expected to end in March 2015), in the custody of Department of Allergy & Immunology, National Center for Child Health and Development. If necessity to analyze these samples in a new study arises, a fresh application will be submitted to the ethics committee within this period. In all other cases, the samples will be disposed of by March 31, 2017 after being heat-sterilized.
22. Actions Related to Flow and Retraction of Samples and Information

① Samples and Flow of personal information

Tachikawa Sougo Hospital
Food challenge test implementation:
Attending physician
Supervisor: Shigenori Kabashima

Challenge test results
(Patient referral document)

Patient introduction
Challenge test implementation request (Patient referral document)

National Center for Child Health and Development
Treatment information data collection: Attending physician
In charge of anonymized information management: Yukihiro

Subject identification table

Blood samples with names
Test results with names

Blood samples with registration no.
Test results with registration no.

Tokushima University
The Institute for Enzyme Research
Department of Molecular Analytical Chemistry
Person in charge: Hiroshi Kido

Hospital laboratory

National Center for Child Health and Development
Research laboratory
Person in charge of sample processing/storage: Kenji Matsumoto

Test results with registration no.

National Center for Child Health and Development
President: Takashi Igarashi
Acting President, Planning & Strategy
Personal information manager
23. Application of Medical Information

As a procedure of this hospital, patient IDs are assigned to patients. Medical information such as onset of food allergy or adverse events will be used as endpoints in the present study.

24. Expected date for submitting the study plan completion report [Form 6]

March 2015

25. References

2. Bright I. Nwaru et al.: Age at the introduction of solid foods during the first year and allergic sensitization at age 5 years. Pediatrics 125, 50-59, 2011
5. Du Tois G. et al.: Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. Journal of Allergy and Clinical Immunology 122, 122, 984

7. Ikematsu Kaori et al.: [Feature of food allergy developed during infancy (1)--relationship between infantile atopic dermatitis and food allergy]. Arerugi 55, 140-150, 2006 (Japanese)

Summary of the Protocol and statistical analysis plan changes

Original (29-Feb-2012): Protocol Version 1.0
Amendment 1 (3-Sep-2012): Protocol Version 1.01
Amendment 2 (30-Jun-2014): Protocol Version 1.02
Amendment 3 (31-Oct-2014): Protocol Version 1.03
Amendment 5 (15-Aug-2015): Protocol Version 1.1 (English)
Amendment 1 (3-Sep-2012) : Protocol Version 1.01

The purpose of this amendment is to:

* Change the egg material to use for the oral food challenge test at 12 months of age from boiled egg white to heated whole egg powder. The reason of this change is to keep a fixed quantity of egg protein and to increase the provocation amount from about one fifth of an egg to half of a boiled egg.

The table below outlines the changes to the protocol:

<table>
<thead>
<tr>
<th>Sections Changed</th>
<th>Original Protocol Version 1.0</th>
<th>Version 1.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 Study Methodology (2) Outline of the trial</td>
<td>Once the subjects turn 12 months of age, an oral food challenge using boiled egg white will be implemented as in-patients to investigate whether or not they have developed an egg allergy.</td>
<td>Once the subjects turn 12 months of age, an oral food challenge using heated whole egg powder will be implemented as in-patients to investigate whether or not they have developed an egg allergy.</td>
</tr>
<tr>
<td>8 Study Methodology (8) Oral food challenge</td>
<td>The infants will be given 1 g, 2 g, and 5 g of boiled egg white, which is boiled for over 20 min, every 30 minutes (total 8 g). The results will be judged as &quot;positive&quot; if the ingestion induces any symptoms such as, hives, nasal discharge, cough, vomiting, abdominal pain, etc. Further intake will be stopped if symptoms appear while the infant is being fed. The results will be judged as &quot;negative&quot; if a total of 20 g of intake does not induce any particular symptom.</td>
<td>The infants will be given 1 g, 2 g, and 4 g of heated whole egg powder every 30 minutes (total 7 g). The results will be judged as &quot;positive&quot; if the ingestion induces any symptoms such as, hives, nasal discharge, cough, vomiting, abdominal pain, etc. Further intake will be stopped if symptoms appear while the infant is being fed. The results will be judged as &quot;negative&quot; if a total of 7 g of intake does not induce any particular symptom.</td>
</tr>
</tbody>
</table>
Amendment 2 (30-June-2014) : Protocol Version 1.02

The purpose of this amendment is to:

- Change the investigators.
- Extent the study period.

The table below outlines the changes to the protocol:

<table>
<thead>
<tr>
<th>Sections Changed</th>
<th>Version 1.01</th>
<th>Version 1.02</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Research Organization (3) Study collaborators</td>
<td>Hirohisa Saito, Kenji Matsumoto, Ichiro Nomura, Kenta Horimukai, Tomoko Suda, Yuki Tsumura, Tetsuo Shoda, Hiroshi Kitazawa, Motoki Yomase, Takahiro Kawaguchi, Kumiko Morita, Machiko Watanabe, Mika Toyomra</td>
<td>Hirohisa Saito, Kenji Matsumoto, Ichiro Nomura, Tomoko Suda, Kumiko Morita, Hisako Komuro, Takeshi Chiba, Aki Gen, Miyuki Hashimoto, Mika Toyomra, Rina Okumura</td>
</tr>
<tr>
<td>3. Research Organization (3) Preparation of Trial powder</td>
<td>Research and Development Department, Kewpie Corporation, 5-13-1, sumiyoshi-cho, futyu-shi, Tokyo Tel: 042-361-0026, Fax: 042-361-6271</td>
<td>Research and Development Department, Kewpie Corporation, Sengawa Kewport, 2-5-7, Sengawa-cho, Chofu-shi, Tokyo Tel: 03-5384-7759</td>
</tr>
<tr>
<td>3. Research Organization (4) Study Office</td>
<td>Shigenori Kabashima, Junko Nakazato</td>
<td>Osamu Natsume, Shigenori Kabashima</td>
</tr>
<tr>
<td>7. Study Duration</td>
<td>Scheduled to start after approval from the ethics committee and end by March 31, 2015.</td>
<td>Scheduled to start after approval from the ethics committee and end by March 31, 2017.</td>
</tr>
</tbody>
</table>
Amendment 3 (31-Oct-2014) : Protocol Version 1.03

The purpose of this amendment is to:
・Describe details of the Data Center, Independent Data Monitoring Committee.
・Make a separate Statistical Analysis Plan to describe details of statistical analysis for this trial by the statistician who joined this trial team before statistical analysis starts.
・Delete one of the secondary endpoints because the flow cytometric analysis costs too much and was thought to be less essential for this trial.

The table below outlines the changes to the protocol:

<table>
<thead>
<tr>
<th>Sections Changed</th>
<th>Version 1.02</th>
<th>Version 1.03</th>
</tr>
</thead>
</table>
| 3. Research Organization (2) Sub-investigators | Osamu Natsume  
Shigenori Kabashima  
Mai Kondo  
Ai Kishino  
Kiwako Yamamoto-Hanada  
Masami Narita  
Hiroshi Kido | Osamu Natsume  
Shigenori Kabashima  
Mai Kondo  
Mayako Saito  
Ai Kishino  
Kiwako Yamamoto-Hanada  
Masami Narita  
Hiroshi Kido |
| 3. Research Organization (5) Person in charge of statistics | (Not described) | Eisuke Inoue  
Clinical Research Data Management Center, Social and Clinical Research Center, National Center for Child Health and Development |
| 3. Research Organization (6) Data Center | (Not described) | Kazuko Okamoto  
Registered Data Management Office, Pediatric Cancer Etiology Clinical Research Center, National Center for Child Health and Development  
Tetsuya Takimoto  
Pediatric Cancer Etiology Clinical Research Center, National Center for Child Health and Development |
| 3. Research Organization | (Not described) | Masayuki Akashi  Pediatrics, Saitama Municipal Hospital  
| (7) Independent Data Monitoring Committee | | Ayako Matsuda  Professor, Department of Hygiene and Public Health, Teikyo University School of Medicine |

<table>
<thead>
<tr>
<th>9. Endpoints</th>
<th>(2) Secondary Endpoints</th>
</tr>
</thead>
</table>
| | ① Serum hen’s egg-specific IgE (ImmunoCAP) at the start of the study, after 3 months, at the end.  
② Serum hen’s egg and other food* specific IgG1, IgG4, and IgA (a novel allergen microarray system, which is equipped with a DLC-coated chip, as previously published (Suzuki K, Anal Chim Acta 2011; 706:321-7.)) at the start of the study, after 3 months, at the end. *other food: milk, wheat, soy, peanut, buckwheat, rice, beef, chicken, house dust, mite, dog, cat.  
③ Flow cytometric analysis of blood cell at the start of the study and at the end.  
④ Serum TARC (thymus and activation-regulated chemokine) at the start of the study, after 3 months, at the end.  
⑤ Salivary IgA, IgG1, and IgG4 measurements (DLC-coated chip) at the start of the study, after 3 months, at the end. |
| | ① Serum hen’s egg-specific IgE (ImmunoCAP) at the start of the study, after 3 months, at the end.  
② Serum hen’s egg and other food* specific IgG1, IgG4, and IgA (a novel allergen microarray system, which is equipped with a DLC-coated chip, as previously published (Suzuki K, Anal Chim Acta 2011; 706:321-7.)) at the start of the study, after 3 months, at the end. *other food: milk, wheat, soy, peanut, buckwheat, rice, beef, chicken, house dust, mite, dog, cat.  
③ Serum TARC (thymus and activation-regulated chemokine) at the start of the study, after 3 months, at the end.  
④ Salivary IgA, IgG1, and IgG4 measurements (DLC-coated chip) at the start of the study, after 3 months, at the end. |

| 13. Statistical Analysis | Difference between the two groups as to primary endpoint is checked to recalculate the sample size and assess safety of the trial. | Details of the statistical analysis are stated in the statistical analysis plan written separately. |
### 13. Statistical Analysis

#### (4) Analysis of Efficacy Endpoints

**Analysis of the primary endpoint**

For the primary comparison, oral food challenge results (positive/negative) of each intervention group at 12 months of age are compared using the Fisher's exact test. FAS is used as the primary analysis set. Secondary analysis is made using PPS. Level of significance is set at 0.05. The 95% confidence interval of the group difference is calculated using binomial distribution.

As secondary evaluation, the primary endpoint is analyzed adjusted for severity of atopic dermatitis evaluated using SCORAD, feeding by breast milk, and history of allergic diseases of both parents are conducted using the logistic regression model.

**Details of the statistical analysis are stated in the statistical analysis plan written separately.**

---

#### (4) Analysis of Efficacy Endpoints

**Analysis of the secondary endpoint**

For the primary comparison, oral food challenge results (positive/negative) of each intervention group at 12 months of age are compared. Comparison between the results of proactive therapy and those of reactive therapy is performed.

Details of the statistical analysis are stated in the statistical analysis plan written separately.

### Other endpoints to evaluate the alteration between before and after the trial are as follows:

1. Serum hen’s egg-specific IgE (egg white and ovomucoid)
2. Serum antigen specific IgG1, IgG4, and IgA
3. Serum CD4+ CD25+ Treg, inflammatory Th2 cell and inflammatory cytokine
4. Salivary IgA, IgG1, and IgG4 concentrations at the start of the study and after 6 months from the trial end.
5. SCORAD score and POEM score

Wilcoxon signed rank test is applied for comparing changes within the group, and Wilcoxon rank sum test is used for comparing the difference between the groups.
as to methods of therapy for atopic dermatitis and trial powders.

| 14. Independent Data and Safety Monitoring Committee | (Not described) | The independent data and safety monitoring committee will monitor the analyses performed as interim analyses and judge the advisability of continuing with the trial when an adverse event has occurred. |
| 22. Handling of Samples after Completion of the Study | Samples such as left-over serum, etc., will be kept anonymous and stored under refrigeration for a period of one year after completion of the study (expected to end in March 2015), in the custody of Department of Allergy & Immunology, National Center for Child Health and Development. | Samples such as left-over serum, etc., will be kept anonymous and stored under refrigeration for a period of one year after completion of the study (expected to end in March 2017), in the custody of Department of Allergy & Immunology, National Center for Child Health and Development. |
Amendment 4 (28-Feb-2015) : Protocol Version 1.1

The purpose of this amendment is to:

- Determine the details of protocol before final analysis.
- Initial plan was to evaluate the difference of outcomes between the patients who were treated with proactive therapy for eczema and those with reactive therapy, however, much more patients than expected desired to receive proactive therapy and many patients who started with reactive therapy was changed to proactive therapy on the way because of insufficient control of eczema. From ethical point of view, we changed our initial plan and decided to do patient’s skin therapy first resulting in giving up to compare the results of proactive therapy and reactive therapy.
- Unnecessary description generated by the above change was deleted.
- Detail description about the method of random allocation was added.
- Describe accurate timing of the blood sampling to measure secondary endpoints
- Post the contents of the Statistical Analysis Plan to the protocol.

The table below outlines the changes to the protocol:

<table>
<thead>
<tr>
<th>Sections Changed</th>
<th>Version 1.03</th>
<th>Version 1.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Research</td>
<td>Hirohisa Saito</td>
<td>Hirohisa Saito</td>
</tr>
<tr>
<td>Organization</td>
<td>Kenji Matsumoto</td>
<td>Kenji Matsumoto</td>
</tr>
<tr>
<td>(3) Study</td>
<td>Ichiro Nomura</td>
<td></td>
</tr>
<tr>
<td>collaborators</td>
<td>Tomoko Suda</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ichiro Nomura</td>
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<td></td>
<td>Kumiko Morita</td>
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<td></td>
<td>Hisako Komuro</td>
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<td>Takeshi Chiba</td>
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<td>Aki Gen</td>
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<td></td>
<td>Miyuki Hashimoto</td>
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<td></td>
<td>Mika Toyomra</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rina Okumura</td>
<td></td>
</tr>
</tbody>
</table>

4. Objective

Also together, patient’s skin condition is continuously observed to evaluate the correlation between the strength and extent of dermatitis and the incidence of egg allergy. Then the preventive effect of onset of egg allergy by the treatment of atopic dermatitis is evaluated. (Deleted)
### 5. Subjects of Study
**Inclusion Criteria**

1. Patients who are 4 or 5 months old at the time of registration.
2. Patients diagnosed with atopic dermatitis according to the Hanifin and Rajka criteria.
3. Patients who will be able to visit the out-patient units of the hospitals for follow up every one or two months, until receiving the oral food challenge test at 12 months of age.
4. Patients who will be able to undertaken the oral food challenge test at 12 months of age.
5. Patients for whom egg intake can be completely eliminated from daily life during intervention, except when given as a trial powder and for the oral food challenge.
6. Patients for whom informed written consent has been obtained from the guardian.

### 8 Study Methodology
**Outline of the trial**

In the meantime, the participants will be treated atopic dermatitis with proactive therapy or reactive therapy based on caregiver’s preference. Participants whose caregiver had no preference of proactive therapy or reactive therapy are allocated to either therapy to equalize the participant number of each therapy group.

### 8 Study Methodology
**Registration and Allocation Participant Assignment**

The participants are randomly allocated to the egg group or the placebo group (1:1) by the data center.
| Study Methodology | The way of proactive therapy for atopic dermatitis is to prevent skin flare proactively by using intermittent topical corticosteroids during the maintenance phase, and that of reactive therapy is to apply topical corticosteroids only when eczema is exacerbated.  
1. Proactive therapy group  
In the first stage of the therapy, participants and their caregivers are given a lecture to educate skin care treatment and application of assignment plan created by the NCCHD involves 100 men and 100 women, while that for the TSH involves 12 men and 12 women. In this study, the Data Center located in the NCCHD is in charge of randomization of the subjects and storage of the clinical data. Therefore, the following procedure is adopted in order to conceal assignment information: at the time of registration of a subject, the Data Center receives information on institution and gender only, and they allocate an assignment code "a" or "b," without knowing the identity of the subject. The assignment is executed based on a computer generated random number table. The result of the assignment is orally communicated to a designated member of the clinical staff, who is the only person who knows which assignment code corresponds to Egg group or Placebo group. This member of staff prepares the trial powder for distribution to subjects without being involved in any other operation of this study. In this way, the subjects, the intervention executors, the outcome evaluators and the Data Center are all blinded from the assignment information.  
The subjects will be treated for atopic dermatitis by allergy specialists. Specifically, proper remission induction will be started, and later, steroid ointment will be applied regularly for a maximum of 2 days/week for maintenance of remission. If remission is maintained successfully, the application of steroid ointment will be gradually weaned off by increasing the application intervals. |
ointments. After their eczema is cleared by application of topical corticosteroids, the therapy moves into the maintenance phase to prevent flare by using emollient and intermittent topical steroids which are tapered gradually based on the physician’s advice. They can visit outpatient units to see the doctors in addition to their scheduled visits.

②reactive therapy group
In the first stage of the therapy participants are and their caregivers are given a lecture to educate skin care treatment and application of ointments. They are advised to use topical steroids when their skin condition is not controlled. They can visit outpatient units to see doctors in addition to their scheduled visits.

The choice of proactive therapy or reactive therapy may not be changed through the trial, so long as caregivers do not want to.

When the skin condition got worse, physicians apply the best treatment at that point and continue the trial.

| 8 Study Methodology | Whether influence of proactive therapy for eczema on percutaneous sensitization of food allergens is different from that of reactive therapy has remained to be elucidated. We respect caregiver’s preference of either eczema therapy and random allocation of each therapy is not applied. Therefore difference of the effect of each eczema therapy on percutaneous sensitization is analyzed by devidint into two subclasses without randomization. | Therefore, atopic dermatitis will be proactively treated to prevent the progress of sensitization during trial intervention. |
## 9. Endpoints

### (2) Secondary Endpoints

1. Serum hen’s egg-specific IgE (ImmunoCAP) at the start of the study, after 3 months, at the end.
2. Serum hen’s egg and other food* specific IgG1, IgG4, and IgA (a novel allergen microarray system, which is equipped with a DLC-coated chip, as previously published (Suzuki K, Anal Chim Acta 2011; 706:321-7.)) at the start of the study, after 3 months, at the end.
   - * other food: milk, wheat, soy, peanut, buckwheat, rice, beef, chicken, house dust, mite, dog, cat.
3. Serum TARC (thymus and activation-regulated chemokine) at the start of the study, after 3 months, at the end.
4. Salivary IgA, IgG1, and IgG4 measurements (DLC-coated chip) at the start of the study, after 3 months, at the end.

## 10. Observation Items

### (4) Symptoms of atopic dermatitis

<p>| | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>the attending physician will evaluate and record the SCORAD (severity scoring of atopic dermatitis) at the start of the trial (4 to 5 months of age) and when implementing the oral food challenge at 12 months of age.</td>
</tr>
<tr>
<td></td>
<td>the attending physician will evaluate and record the SCORAD (severity scoring of atopic dermatitis) at the time of initial medical examination and when implementing the oral food challenge at 12 months of age.</td>
</tr>
</tbody>
</table>
### 13. Statistical Analysis

#### (3) Data Handling

<table>
<thead>
<tr>
<th>Handling of withdrawal case data</th>
<th>Handling of missing values</th>
</tr>
</thead>
</table>
| ① As to FAS, results of participant’s blood examinations and an oral food challenge test are used as outcomes even if they were carried out before the end of the trial. If those data do not exist, outcome data of the case are handled as missing values.  
② As to PPS, results of participant’s blood examinations and an oral food challenge test are used as outcomes even if they were carried out before the end of the trial. If those data do not exist, outcome data of the case are handled as missing values.  
③ Data fixed before the release of blinded assignment should be used as outcomes to be analyzed.  
Supplementation of missing values of oral food challenge tests. Handling after violation matters
If the data of withdrawal cases are recognized as "missing values", their results are handled as positive in the oral food challenge tests. | Regardless of the reason, missing values of the primary endpoint is handled as positive.  
Missing values of the secondary endpoints is excluded from the analysis.  
Handling methods for unexpected missing data is decided after discussions between trial statisticians. |

Handling of outliers
If there are outliers that ought to be excluded from the analysis, the values as well as the reason for exclusion must be mentioned in the analysis report.

#### (4) Level of Significance

All comparisons is made using the two sided test, with level of significance per comparison being 0.05. The confidence coefficient for confidence interval is 0.95.

#### (5) Frequency

- Integer
- Proportion: One decimal place
- Maximum, Minimum: same decimal place as the intended variable
- Mean, Median: one less decimal point than the intended variable
- Standard deviation: two less decimal point than the intended variable
- P value: 4 decimal place

#### (6) Software to be Used

All statistical analyses is performed using R version 3.1.0.
Cases whose diary was lost or blank: the result of the oral food challenge test is used as primary endpoint in FAS and PPS. Missing values caused by withdraw and positive results of endpoint caused by violation matter are recorded in the analytical procedure, and those are excluded in the data summary. The other handling of cases and data not described in this protocol such as data fixation whether they should be included or excluded are determined after discussion among statistician and trial investigators. The key open is performed after data fixation.

<table>
<thead>
<tr>
<th>13 Analysis (4) Method of Analysis</th>
<th>Protocol violations</th>
<th>Protocol violations</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 Analysis (6) Interim Analyses</td>
<td>Details of the statistical analysis is stated in the statistical analysis plan written separately.</td>
<td>Ø Point estimates for the primary endpoint for two groups are calculated. Comparison is not made based on the hypothesis test. Ø Conditional power is calculated using the interim results of the trial to evaluate the probability of success of the study. This evaluation is carried out by the Independent Data and Safety Monitoring Committee. Ø If the estimated intervention effect significantly away from the value used in the sample size calculation, the sample size is re-estimated. However, the decision regarding continuation of the trial by changing the protocol is taken by the Independent Data and Safety Monitoring Committee.</td>
</tr>
<tr>
<td>Protocol violations</td>
<td>In each group, the number of withdrawals are summarized. This description is done separately for FAS and PPS.</td>
<td>Protocol violations The number of subjects in each group, number of drop-outs, and the number of withdrawals is summarized. Also, reasons for dropping-out/withdrawal are summarized. This</td>
</tr>
<tr>
<td>(7) Descriptive statistics</td>
<td>13 Analysis (4) Method of Analysis ↓ 13Analysis (7) Descriptive statistics 3 Summary of background information</td>
<td>Description is done separately for FAS and PPS. Summary of background information For each groups, gender, nutrition during infancy, history of allergic diseases of both parents, serum immunological test readings, POEM values, diagnosis of oral food challenge, serum TARC, etc. is summarized. The method for description: For nominal data, the number of subjects and proportion (%) are used. For continuous data, mean and standard deviation, and median, 25% value, 75% value, minimum value, and maximum value are used.</td>
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<td>---------------------------</td>
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</tr>
<tr>
<td>13 Analysis (4) Method of Analysis Analysis of the Primary Endpoint ↓ 13Analysis (8) Analysis of Efficacy Endpoints - 1 Analysis of the Primary Endpoint</td>
<td>For the primary comparison, oral food challenge results (positive/negative) of each intervention group at 12 months of age are compared. Details of the statistical analysis are stated in the statistical analysis plan written separately. For the primary comparison, oral food challenge results (positive/negative) of each intervention group at 12 months of age are compared using the chi-squared test. Yates continuity correction is not performed. FAS is used as the primary analysis set. Secondary analysis is made using PPS. Level of significance is set at 0.05. Point estimates of the negative test are calculated for each intervention group and its 95% confidence interval is obtained using the Clopper-Pearson method, which uses a binomial distribution. Similarly, point estimates of the group difference as well as its 95% confidence interval also are calculated. As secondary evaluation, analyses adjusted for severity of atop dermatitis evaluated using SCORAD, history of allergic diseases of both parents, and baby food initiation time are conducted using the logistic regression model. In addition, the analysis using all items in the</td>
<td></td>
</tr>
</tbody>
</table>
above related to the primary endpoint as adjustment factors are confirmed the result of main comparison.

<table>
<thead>
<tr>
<th>13 Analysis</th>
<th>Comparison between the results of proactive therapy and those of reactive therapy is performed. Details of the statistical analysis are stated in the statistical analysis plan written separately.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(4) Method of Analysis</td>
<td>Ø In relation to the following endpoints, Wilcoxon signed rank test is used for comparing changes within the group, and Wilcoxon rank sum test is used for comparing the group difference for the trial intervention. FAS is used as the analysis set. PPS also is used for the secondary analysis. Level of significance is set at 0.05.</td>
</tr>
</tbody>
</table>
| Analysis of the Secondary Endpoint | § Serum antigen-specific IgE, IgG1, IgG4, and IgA at the enrollment, at 9 months of age, and at 12 months of age  
§ Serum TARC (thymus and activation-regulated chemokine) at the time of initial medical examination, at 9 months of age, and at 12 months of age  
§ Salivary antigen-specific IgA, IgG1, and IgG4 at 6, 12, and 18 months of age |
Amendment 5 (15-Aug-2015) : Protocol Version 1.1 (English)

The purpose of this amendment is to:
* translate the protocol from Japanese to English

<table>
<thead>
<tr>
<th>Sections Changed</th>
<th>Version 1.1 (Japanese)</th>
<th>Version 1.1 (English)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>Japanese</td>
<td>English</td>
</tr>
</tbody>
</table>
Protocol for a Study on the Prevention of Onset of Egg Allergy in Infants with Atopic Dermatitis

PETIT study (Prevention of Egg allergy with Tiny amount InTake)

Clinical Protocol

Protocol Version 1.0 (Japanese) (Created February 29, 2012)
Protocol Version 1.1 (English) (Translated August 15, 2015)
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1. Research Title
   Protocol for a study on prevention of onset of egg allergy in infants with atopic dermatitis
   (PETIT study (Prevention of Egg allergy with Tiny amount InTake))

2. Principal Investigator
   Yukihiro Ohya  Chief Physician - Division of Allergy, Department of Medical Specialties, National Center for Child Health and Development

3. Research Organization
   (1) Principal Investigator
      Shall be responsible for managing and supervising the present study.
      Yukihiro Ohya  Chief Physician - Division of Allergy, Department of Medical Specialties, National Center for Child Health and Development

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      Director, Department of Pediatrics, Tachikawa Sougo Hospital, Kenseikai
      Junko Nakazato  Physician - Division of Allergy, Department of Medical Specialties, National Center for Child Health and Development
      Mai Kondo  Physician - Division of Allergy, Department of Medical Specialties, National Center for Child Health and Development
      Mayako Saito  Physician - Division of Allergy, Department of Medical Specialties, National Center for Child Health and Development
      Ai Kishino  Physician - Division of Allergy, Department of Medical Specialties, National Center for Child Health and Development
      Kiwako Yamamoto-Hanada  Physician - Clinical Study Member - Division of Allergy, Department of Medical Specialties, National Center for Child Health and Development
      Masami Narita  Physician - Division of Allergy, Department of Medical Specialties, National Center for Child Health and Development
      Hiroshi Kido  Center Head, The Institute for Enzyme Research, Tokushima University

   (3) Study collaborators
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      Kenji Matsumoto  Division Head, Department of Allergy & Immunology, National Center for Child Health and Development

   (3) Preparation of Trial powder
      Responsibility for manufacturing and packaging, blinding, and maintaining the quality of trial powder, until it is sent to the research implementing institution.
      Research and Development Department, Kewpie Corporation
      Sengawa Kewport, 2-5-7, Sengawa-cho, Chofu-shi, Tokyo  Tel: 03-5384-7759
      Person in charge: Mamoru Kimura

   (4) Study Office
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      Shigenori Kabashima,  Division of Allergy, Department of Medical Specialties, National Center for Child Health and Development
      Junko Nakazato  Division of Allergy, Department of Medical Specialties, National Center for Child Health and Development

   (5) Person in charge of statistics
      Eisuke Inou  Clinical Research Data Management Center, Social and Clinical Research Center, National Center for Child Health and Development

   (6) Data Center
      Kazuko Okamoto  Registered Data Management Office, Pediatric Cancer Etiology Clinical Research Center, National Center for Child Health and Development

4. Background and Objectives of the Study

Background

Atopic dermatitis, food allergies, and bronchial asthma are three allergy diseases that are known to have a high prevalence rate in infants, greatly affecting the quality of life (QOL) of those affected. Particularly, food allergies in infants have been increasing in recent years and are turning into a big problem. The onset of food allergies is most common in infants up to one year of age. Most food allergies during this stage are caused by hen’s eggs, cow’s milk, and wheat. Owing to the high daily intake frequency as well as the high nutritional value of these products, a restriction on their intake can greatly affect their QOLs. Moreover, accidental ingestion of an allergenic food can cause serious symptoms such as anaphylaxis, causing the affected infant and their family a great deal of anxiety.

There have been attempts to eliminate allergenic food from the diets of children as well as those of pregnant and nursing women in order to prevent the onset of food allergy. However, such attempts have been consistently reported to have failed. In its publication in the year 2000, the American Academy of Pediatrics (AAP) advocated for delayed introduction of baby foods known to be highly allergenic, but in 2008, it reversed these guidelines. Instead, recently there have been a few reports showing that an early introduction to highly allergenic foods lowers the frequency of food allergies1) 2).

On the other hand, a prospective cohort study implemented at our center has revealed a strong association between food allergy and atopic dermatitis. Children who contracted eczema within first 6 months of age showed an odds ratio of 10-20 for contracting a food allergy by the age of 1 ~ 3 compared to children who did not contract eczema3).

Currently, a new idea known as the dual-allergen-exposure hypothesis is gaining attention as an explanation for this phenomenon4) 5). As per this hypothesis, patients with atopic dermatitis have a disrupted skin barrier that allows exposure to food proteins, which results in the development or strengthening of allergy antigen sensitization. On the other hand, the ingestion of food proteins induces an immunological tolerance. This hypothesis suggests that immunological tolerance by early introduction to antigenic food proteins through baby foods, or inducing preventing percutaneous sensitization by maintaining the skin in a healthy state during infancy may have a preventive effect on the onset of food allergies.

Objective

In the present study, we shall focus on hen’s egg, described in a preceding retrospective study as the most common food that leads to onset of food allergy during infancy. The study targets are 4~5-month-old infants with atopic dermatitis, as they are considered to be a high-risk group for developing food allergies. After daily oral administration of heated whole egg powders to a large number of infants for 6 months (from the time they are 6 months old and start baby food until they turn 1), we will implement an oral food challenge once they have completed 12 months of age to investigate whether or not they have developed an egg allergy. In this way, we will
investigate the efficacy of introducing egg from an early stage of baby food as a preventive therapy against the onset of immediate-type egg allergy.

5. Subjects of Study

Patients with atopic dermatitis who meet all of the following inclusion criteria and do not fall under any of the exclusion criteria.

Inclusion Criteria

① Patients who are 4 or 5 months old at the time of registration.
② Patients diagnosed with atopic dermatitis according to the Hanifin and Rajka criteria.
③ Patients for whom egg intake can be completely eliminated from daily life during intervention, except when given as a trial powder and for the oral food challenge.
④ Patients for whom informed written consent has been obtained from the guardian.

Exclusion Criteria

① Patients born before 37 weeks of gestation.
② Patients who have already been introduced to hen’s eggs.
③ Patients who have a history of immediate-type egg allergy.
④ Patients suspected to have a particular food allergy other than immediate-type response.
⑤ Patients judged by the attending physician as having a serious coexisting disease that could cause hindrance in the implementation of this study.
⑥ Patients judged to be medically unfit for participation in this study by the attending physician.
6. Target Sample Size and Basis for Establishment Thereof

Target Sample Size

- Egg group: 100 infants
- Placebo group: 100 infants
- Total: 200 infants

Basis for Establishment

According to studies\(^{(1,6,7)}\) on the incidence rate of immediate-type allergy in infants with atopic dermatitis reported so far, a nation-wide prevalence study\(^{(8)}\) on atopic dermatitis and immediate-type food allergies, as well as the data obtained from a birth cohort study (unpublished) being implemented at this center, the incidence rate of immediate-type hen’s egg allergy in 1 year old infants with atopic dermatitis is estimated to be 7% when intervened by intake of egg, and 20% in case of placebo intake. In this study, the sample size needed to detect the difference with 80% power using a two-sided test (5%) is 92 subjects in each group. There will be 100 subjects in each group, presupposing a dropout rate of about 10%.

7. Study Duration

Scheduled to start after approval from the ethics committee and end by March 31, 2017.

Hanifin and Rajka Diagnostic Criteria for Atopic Dermatitis

- Must have 3 or more basic features
  - Pruritus
  - Typical morphology and distribution
    - Flexural lichenification or linearity in adults
    - Facial and extensor involvement in infants and children
  - Chronic or chronically-relapsing dermatitis
  - Personal or family history of atopy (asthma, allergic rhinitis, atopic dermatitis)

- Prus 3 or more minor features:
  - Xerosis
  - Ichthyosis/palmar hyperlinearity/keratosis pilaris
  - Immediate (type I) skin test reactivity
  - Elevated serum IgE
  - Early age of onset
  - Tendency toward cutaneous infections (esp. Staph. Aureus and Herpes simplex)/impaired cell-mediated immunity
  - Tendency toward non-specific hand or foot dermatitis
  - Nipple eczema
  - Cheilitis
  - Recurrent conjunctivitis
  - Dennis-Morgan infraorbital fold
  - Keratoconus
  - Anterior subcapsular cataracts
  - Orbital darkening
  - Facial pallor/facial erythema
  - Pityriasis alba
  - Anterior neck folds
  - Itch when seating
  - Intolerance to wool and lipid solvents
  - Perifollicular accentuation
  - Food intolerance
  - Couse influenced by environmental/emotional factors
  - White dermographism/delayed blanch

8. Study Methodology

(1) Type of trial
A double-blind, parallel group, stratified, randomized controlled trial

Basis for Establishment
In the present study, a hen’s egg daily intake group and a placebo group will be formed and compared in order to investigate the safety and efficacy of food allergy preventive therapy, in which egg will be given in early phase of baby food.

We decided to conduct a double-blind study to eliminate any bias on the part of the subjects or the researchers during the evaluation of safety and efficacy of hen’s egg intake. Moreover, placebo control is necessary as it is impossible to accurately estimate the incidence rate of immediate-type allergies in infants.

The burden of daily intake of trial powder for a period of 6 months on the subject and their family is considered to be permissible. Moreover, currently there is a tendency to introduce eggs in baby food once the infant is 7-8 months old, with it being common to prescribe long-term elimination of eggs as a part of treatment, particularly for infants with atopic dermatitis. In the present study, eggs will be completely eliminated for the placebo group until the subjects complete 12 months of age, which is considered to be within the permissible range.

(2) Outline of the Trial

From among the 4-5 month old infants diagnosed with atopic dermatitis according to the Hanifin and Rajka criteria, those for whom consent for participation in the study is obtained from the guardians will be registered. Subjects will be randomly assigned to either the "Egg group" who will ingest hen’s eggs, or the "Placebo group" who will ingest placebo. Once the subject turns 6 months of age, 300 mg/day of trial powder composed of 50 mg of heated whole egg powder, 100 mg of pumpkin powder, and 150 mg of glucose powder will be started for the Egg group, whereas trial powder composed of pumpkin powder (150 mg/day) and glucose powder (150 mg/day) will be started for the placebo group. For the sake of safety, both groups will be given their first intake at the hospital as outpatients. The same amount of intake per day will be continued until they are 9 months old. Once the subject turns 9 months of age, the intake amount will be increased to 500 mg/day of trial powder composed of
heated whole egg powder 250 mg and glucose powder 250 mg for the Egg group, and trial powder composed of pumpkin powder 250 mg and glucose powder 250 mg for the Placebo group. For the sake of safety, both groups will be given the first increased amount of trial powder as outpatients at the hospital. Hereafter, the same amount of intake will be continued until they are 12 months old. Once the subjects turn 12 months of age, an oral food challenge using heated whole egg powder will be implemented as in-patients to investigate whether or not they have developed an egg allergy.

In the meantime, the participants will be treated for atopic dermatitis by allergy specialists. Specifically, proper remission induction will be started, and later, steroid ointment will be applied regularly for a maximum of 2 days/week for maintenance of remission. If remission is maintained successfully, the application of steroid ointment will be gradually weaned off by increasing the application intervals.

**Basis for Establishment**

The initial intake quantity of heated whole egg powder was established as 50 mg (equivalent to 0.2 g of whole egg boiled in hot water for 15 minutes) after referring to previously reported allergen thresholds for hen’s egg, and symptom provocation thresholds established in food challenge tests conducted by previous studies using egg powder ("Study on oral desensitization treatment including rush induction using heated egg powder" and "Study on oral desensitization treatment with slow induction using heated egg powder") and after considering its quantifiable feasibility as food.

Pumpkin powder was selected as the placebo powder as it has extremely low allergenicity, is widely used as initial-stage baby food, and is similar to boiled egg powder in terms of color and texture. Glucose is also added to improve the liquidity of the powder.

**(3) Registration and Allocation**

**Registration Process**

After obtaining consent for participation in the study, the physician-in-charge will confirm that the subjects meet all the subject inclusion criteria and do not fall under the exclusion criteria, after which physicians will enter the anonymization numbers, registration date, date of birth, and gender in the case registration table, and will contact the data center at the address given below. At the data center, the subjects will be stratified on the basis of gender and registration institution, and equal number of subjects will be allocated to the Egg group and the Placebo group.

[Contact address for case registration]
Clinical Research Center, National Center for Child Health and Development (Hereinafter referred to as the "Data Center")
Person in charge: Tetsuya Takimoto, Division Chief, Department of Clinical Research Promotion
Eligibility Verification

The Data Center will verify the eligibility of the target group.
1) Subjects shall not be registered if the registration table is not filled in properly.
2) Once registered, the subjects shall not be deleted.
3) In case of double registration, the information registered the first time shall be used.

Participant Assignment

The subjects are randomly assigned to the Egg group or the Placebo group in a ratio of 1:1. Assignment is carried out using the block randomization method. The block size is four; the subjects are stratified into 4 groups based on the institution (Tachikawa Sougo Hospital: TSH, National Center for Child Health and Development: NCCHD) and gender. The assignment plan created by the NCCHD involves 100 men and 100 women, while that for the TSH involves 12 men and 12 women. In this study, the Data Center located in the NCCHD is in charge of randomization of the subjects and storage of the clinical data. Therefore, the following procedure is adopted in order to conceal assignment information: at the time of registration of a subject, the Data Center receives information on institution and gender only, and they allocate an assignment code "a" or "b," without knowing the identity of the subject. The assignment is executed based on a computer generated random number table. The result of the assignment is orally communicated to a designated member of the clinical staff, who is the only person who knows which assignment code corresponds to Egg group or Placebo group. This member of staff prepares the trial powder for distribution to subjects without being involved in any other operation of this study. In this way, the subjects, the intervention executors, the outcome evaluators and the Data Center are all blinded from the assignment information.

(4) Atopic Dermatitis Treatment Method

The subjects will be treated for atopic dermatitis by allergy specialists. Specifically, proper remission induction will be started, and later, steroid ointment will be applied regularly for a maximum of 2 days/week for maintenance of remission. If remission is maintained successfully, the application of steroid ointment will be gradually weaned off by increasing the application intervals.

Basis for Establishment

According to the dual-allergen-exposure hypothesis mentioned earlier, stimulation with dietary protein via skin strengthens sensitization to food allergens, but the degree of sensitization may vary depending upon the skin condition. Therefore, atopic dermatitis will be proactively treated to prevent the progress of sensitization during trial intervention.

(5) Amount, Period, and Method of Trial powder Intake for Each Group

Intake Amount and Intake Period

① Egg group

[From 6 months of age until 8 months of age]
Mixture of 50 mg of heated whole egg powder, 100 mg of pumpkin powder, and 150 mg of glucose powder

[From 9 months of age until 11 months of age]
Mixture of 250 mg of heated whole egg powder and 250 mg of glucose powder
Storage method Cryopreserved

② Placebo group
[From 6 months of age until 8 months of age]
Mixture of 150 mg of pumpkin powder and 150 mg of glucose powder
[From 9 months of age until 11 months of age]
Mixture of 250 mg of pumpkin powder and 250 mg of glucose powder
Storage method Cryopreserved

For both the groups, the trial powder packets to be consumed each day will be provided beforehand to the guardians, and feed of 1 packet per day will be started once the infant is 6 months old ± 2 weeks. The daily intake will be continued for a period of 6 months, that is, when the infant is between 6 ~ 11 months of age. During this period, the quantity of trial powder will be increased once the infant is 9 months old ± 2 weeks. For the sake of safety, the first feed at 6 months and subsequent quantity increase at 9 months will be given at the hospital as an outpatient.

Intake Method
The trial powder intake for both the groups will be 1 packet per day. There is no particular feeding method and the trial powder can be given separately or by mixing with other food like porridge; however, it must not be heated at over 100°C, for example, cooked in the oven etc.

Discontinuation of Intake when Unwell
The trial powder will not be given when the infant is unwell, for example with gastroenteritis, fever, etc. If the intake of test sample has been discontinued for more than 3 days due to an illness, when restarting, the trial powder quantity will be cut down to half the prescribed amount for the initial 2 days.

Basis for Establishment
The trial powder must not be cooked at over 100°C as the hen’s egg protein undergoes denaturation if processed at high temperatures, such as in the oven, thereby weakening its properties as a food allergen, and may therefore hinder the development of the immunological tolerance expected through the introduction of continued intake of the trial powder.
Even normally well-tolerated food may induce allergic symptoms when a child is physically unwell with gastroenteritis, etc. Therefore, if the intake of trial powder is temporarily discontinued owing to an illness, it must be cautiously restarted by reducing the intake quantity to half.

(6) Trial powder
Overview of trial powder is given below.
Heated whole egg powder
Preparation method: Heated (95°C for 15 minutes), pulverized, heat sterilized, spray-dried
Additives: None
Egg production area: Japan
Manufacturer: Kewpie Corporation
Powder packaging: Glip Co., Ltd.
Warranty period from date of manufacture: 6 months when stored under -20°C in hermetically sealed individual packets
Microbiological standards: General viable bacterial count 5000/g or less, fungi/yeast 300/g or less
E. Coli/Staphylococcus aureus/Salmonella negative
Contamination: The trial powder will be checked for soybean/milk/wheat contamination using FASTKIT Elisa Ver. II (antigen measurement kit manufactured by Nipponham).
The Kewpie Corporation which handles the manufacturing and individual packaging offers guarantee for the specifications mentioned above.

Pumpkin Powder (Vegetable fine powder – KABOTYA®)
Preparation method: Heat and steam sterilized, desiccated, jet-stream pulverized
Additives: None
Pumpkin production area: Japan
Manufacturer: Mikasa Sangyo Co., Ltd.
Guarantee period from date of manufacture: 24 months when stored under normal temperature in a cool dark place
Contamination: As per the Food Sanitation Law, 25 allergic substances cannot be manufactured by pulverization inside the same factory.
Mikasa Sangyo that handles the manufacturing offers guarantee for the specifications mentioned above.

Glucose Powder (Showa anhydrosugar powder)
Additives: None
Production area: Japan
Manufacturer: Showa Co., Ltd.
Quality guarantee period from date of manufacture: One year from manufacture when stored at normal temperature
Contamination: As per the Food Sanitation Law, 25 allergic substances cannot be manufactured by pulverization inside the same factory.

(7) Restrictions
Intake of eggs and food products containing eggs is prohibited when the infant is between 6-12 months of age and is being fed the trial powder. The nursing mother is not prohibited from consuming eggs and egg-containing foodstuff even when the infant is being breast-fed.
[Basis for Establishment] Intake is prohibited as intake of egg-containing foodstuffs by the subject during the trial period may influence the efficacy evaluation of the study. Even if the nursing mother consumes egg-containing foodstuffs, only a minuscule amount of egg protein is secreted in the breast milk, which may have a much smaller
effect on efficacy evaluations of the study therapy. Additionally, complete elimination of egg-containing foodstuff from the mother's diet may greatly affect her life, and therefore has not been restricted.

(8) Oral food challenge

Oral food challenge Implementation Period

The oral food challenge will be implemented between 2 weeks before the infant's 1st birthday and 4 weeks after it.

Overview of Oral food challenge

The oral food challenge will be implemented as an open method, in which the contents of the food to be ingested will be known to the tester and the subjects. The infants will be given 1 g, 2 g, and 4 g of heated whole egg powder every 30 minutes (total 7 g). The results will be judged as "positive" if the ingestion induces any symptoms such as, hives, nasal discharge, cough, vomiting, abdominal pain, etc. Further intake will be stopped if symptoms appear while the infant is being fed. The results will be judged as "negative" if a total of 7 g of intake does not induce any particular symptom.

The oral food challenge will be implemented at NCCHD or Tachikawa Sougo Hospital. The same procedure will be used at both hospitals. The participants decide which hospital they will take the challenge test in.

[Basis for Establishment]

A double-blind, placebo-controlled oral food challenge is the gold standard for diagnosis of immediate-type food allergies. However, the one-year-old subjects of the present study are unlikely to have psychogenic reactions. Therefore, an open method, which can be implemented easily and has less loads on the subjects, is expected to give a highly credible assessment. Therefore, an open method, which has less loads on the subjects, is expected to give a highly credible assessment.

9. Endpoints

(1) Primary Endpoint

Negative rate of diagnosis from oral food challenge implemented on 12 months olds (open method)

[Basis for Establishment]

A oral food challenge (double-blind trial) is the gold standard when it comes to diagnosis of immediate-type food allergy. As mentioned earlier, since the subjects being evaluated are 1-year old infants with negligible psychogenic reactions, an open oral food challenge is considered to be adequately reliable. Additionally, an open method reduces the loads on the subjects by half as compared to conducting the test as a double blind method.

(2) Secondary Endpoints

① Serum hen's egg-specific IgE (ImmunoCAP) at the time of enrollment for trial, at 9 months of age, and at 12 months of age

② Serum hen's egg specific IgG1, IgG4, and IgA (a novel allergen microarray system, which is equipped with a DLC-coated chip, as previously published (Suzuki K, Anal Chim Acta 2011;706:321-7.)) at the
time of enrollment for trial, at 9 months of age, and at 12 months of age

③ Serum TARC (thymus and activation-regulated chemokine) at the time of initial medical examination, at 9 months of age, and at 12 months of age

④ Salivary IgA, IgG1, and IgG4 measurements (DLC-coated chip) at 6, 12, and 18 months of age

[Basis for Establishment]

Antigen-specific IgE is an index of sensitization to dietary proteins that cause food allergies.

IgG1, IgG4, and IgA are important factors of immunoreaction in the onset of food allergies. A blood examination will be performed in order to assess immunological change during the intervention and also to evaluate sensitization to other foods. Association with immunological tolerance will be investigated by comparing specific salivary IgA, IgG1, and IgG4 antibodies with serum IgE.

10. Observation Items

(1) Background of participants

The following items will be investigated before the registration process.

① Gender, date of birth, gestational age, weight, patient's initials, clinical records number

② Mode of nutrition (exclusive breast feeding, mixed feeding, or bottle feeding)

③ Complication or history of food allergy

④ Regular medication

⑤ Other coexisting disease

⑥ Family history

(2) Status of intake of trial powder as well as compliance with antigen eliminated diet

At every visit as an outpatient, the logs maintained by the guardian will be checked.

(3) Confirmation of adverse events

At every visit as an outpatient, the logs maintained by the guardian are checked.

(4) Symptoms of atopic dermatitis

The guardians will evaluate and record the symptoms related to skin findings observed by them once a week using POEM (Patient-Oriented Eczema Measure) between 6 months ~ 12 months of age. Additionally, the attending physician will evaluate and record the SCORAD (severity scoring of atopic dermatitis) at the time of initial medical examination and when implementing the oral food challenge at 12 months of age.

(5) Observation and Examination Schedule

The above observations and examinations will be implemented based on the following examination schedule table.
Implementation items / implementation period | First visit | Enroll (when 4~5 months old) | 6 months old<sup>1</sup> | Regular checkups every 1~2 months | 9 months old<sup>2</sup> | Regular checkups every 1~2 months | 1 year old<sup>3</sup>
--- | --- | --- | --- | --- | --- | --- | ---
Consent acquisition |  |  | o |  |  |  |  
Investigation of subjects’ background |  |  | o |  |  |  |  
Assignment to the Egg group / Placebo group |  |  | o |  |  |  |  
Blood collection | o | o | o | o | o | o | 
Saliva |  |  | o |  |  |  |  
Dermatitis treatment | Start |  |  |  |  |  | Complete (continued if required) 
Skin findings (SCORAD) assessment | o | o |  |  |  |  |  
Skin findings (POEM) assessment | o | o | o | o | o | o |  
Test food intake as outpatient | o | o |  |  |  |  |  
Test food intake |  |  |  |  |  |  | Complete 
Test food intake status check | o | o | o | o | o | o |  
Adverse event confirmation | o | o | o | o | o | o |  
Food challenge test (egg albumin intake) |  |  |  |  |  |  | o 
Questionnaire |  |  |  |  |  |  | o 

1) 2) Age in months ± 2 weeks
3) Age in months ± 3 weeks

11. Withdrawal Criteria

Any subject falling under any of the following withdrawal criteria will be withdrawn from the study

① Guardian of the subject has refused participation in the trial or has revoked the consent.

② The trial powder causes symptoms of immediate-type allergy during the trial period. Symptoms of immediate-type allergy are adverse events occurring within 120 minutes of consuming the trial powder, manifesting in the form of skin symptoms, respiratory symptoms, mucous membrane symptoms, gastrointestinal symptoms, or abdominal symptoms. Additionally, if continuation in the trial becomes difficult due to other adverse event.

③ If continuation of the intervention becomes difficult due to aggravation of complications.

④ If judged as not fulfilling the eligibility criteria after the registration.

⑤ The subject stops to visit during the trial and becomes untraceable.

⑥ The entire trial has been discontinued.

⑦ The doctor finds it appropriate to discontinue the trial for some other reasons.

12. Handling of an adverse event

① Dealing with the subjects in case of an adverse event

The study representative or the sub-investigators will immediately take appropriate medical measures upon observing an adverse event.
② Reporting of serious adverse events

A serious adverse event is an unfavorable event fitting any of the following definitions provided in points ① ~ ⑤.

① Death
② Life-threatening event
③ Event requiring hospitalization or extension of hospitalization period
④ Event leading to disability or impairment
⑤ Later-generation congenital disease or abnormality

The adverse events that must be reported include; all serious adverse events that occur during the trial participation, serious adverse events suspected to be associated with the trial powder after completion (discontinuation) of study therapy, as well as other adverse events for which medical reporting is judged to be necessary by the study representative. However, this does not include hospitalization for the purpose of the oral food challenge scheduled for the trial.

Upon observing the occurrence of the above-mentioned adverse events, the study representative will promptly send a written report to the center head and the ethics committee through the ethics committee office, to seek their review and judgment regarding advisability of continuation with the trial.

13. Statistical Analysis
(1) Analysis Set

FAS (Full Analysis Set)
FAS consists of all randomized subjects who take at least one trial powder

PPS (Per Protocol Set)
PPS consists of all randomized subjects who take trial powder and fulfill the following criteria.

① Fulfill all the inclusion criteria and do not meet the exclusion criteria.
② Take trial powder for at least 130 days.
③ Successfully eliminated hen’s-egg containing foodstuff from the diet, except for trial powder, with accidental ingestion during trial limited to a maximum of 3 times.

The primary analysis set for efficacy and safety analyses is FAS and secondary analysis set is PPS. Handling of FAS and PPS is determined for each endpoint.

(2) Definition of case categorization

Eligible cases: Cases that fulfill all the inclusion criteria and do not meet the exclusion criteria
Withdrawal cases: Cases that are withdrawn from the trial due to the criteria mentioned in Section 11

(3) Data Handling

• Handling of missing values
Regardless of the reason, missing values of the primary endpoint is handled as positive.

Missing values of the secondary endpoints is excluded from the analysis.

Handling methods for unexpected missing data is decided after discussions between trial statisticians.

- Handling of outliers
  - If there are outliers that ought to be excluded from the analysis, the values as well as the reason for exclusion must be mentioned in the analysis report.

(4) Level of Significance

All comparisons is made using the two sided test, with level of significance per comparison being 0.05. The confidence coefficient for confidence interval is 0.95.

(5) Software to be Used

All statistical analyses is performed using R version 3.1.0.

(6) Interim Analyses

One interim analysis is planned in this study.

- Timing
  - One year after the beginning of the study or when 100 subjects completed this study.

- Analysis
  - Point estimates for the primary endpoint for two groups are calculated. Comparison is not made based on the hypothesis test.
  - Conditional power is calculated using the interim results of the trial to evaluate the probability of success of the study. This evaluation is carried out by the Independent Data and Safety Monitoring Committee.
  - If the estimated intervention effect significantly away from the value used in the sample size calculation, the sample size is re-estimated. However, the decision regarding continuation of the trial by changing the protocol is taken by the Independent Data and Safety Monitoring Committee.

(7) Descriptive statistics

- Protocol violations
  - The number of subjects in each group, number of drop-outs, and the number of withdrawals is summarized. Also, reasons for dropping-out/withdrawal is summarized. This description is done separately for FAS and PPS.

- Status of intake of trial powder
  - The number of days of the trial powder consumed is summarized.
-3 Summary of background information
  ● For each groups, gender, nutrition during infancy, history of allergic diseases of both parents, serum immunological test readings, POEM values, diagnosis of oral food challenge, serum TARC, etc. is summarized.
  ● The method for description:
    ➢ For nominal data, the number of subjects and proportion (%) are used.
    ➢ For continuous data, mean and standard deviation, and median, 25% value, 75% value, minimum value, and maximum value are used.

(8) Analysis of Efficacy Endpoints
-1 Analysis of the Primary Endpoint
  For the primary comparison, oral food challenge results (positive/negative) of each intervention group at 12 months of age is compared using the chi-squared test. Yates continuity correction is not performed. FAS is used as the primary analysis set. Secondary analysis is made using PPS. Level of significance is set at 0.05. Point estimates of the negative test is calculated for each intervention groups and its 95% confidence interval is obtained using the Clopper-Pearson method, which uses a binomial distribution. Similarly, point estimates of the group difference as well as its 95% confidence interval also is calculated.
  As secondary evaluation, analyses adjusted for severity of atopic dermatitis evaluated using SCORAD, history of allergic diseases of both parents, and baby food initiation time are conducted using the logistic regression model.

-2 Analyses of Secondary Endpoints
  ➢ In relation to the following endpoints, Wilcoxon signed rank test is used for comparing changes within the group, and Wilcoxon rank sum test is used for comparing the group difference for the trial intervention. FAS is used as the analysis set. PPS also is used for the secondary analysis. Level of significance is set at 0.05.
    ◦ Serum antigen-specific IgE, IgG1, IgG4, and IgA at the enrollment, at 9 months of age, and at 12 months of age
    ◦ Serum TARC (thymus and activation-regulated chemokine) at the time of initial medical examination, at 9 months of age, and at 12 months of age
    ◦ Salivary antigen-specific IgA, IgG1, and IgG4 at 6, 12, and 18 months of age

(9) Analysis of Safety Endpoints
  Adverse events is classified into serious and not serious, the name of adverse event, number of cases in which they appeared, and frequency of occurrence is calculated. Name of adverse reaction, number of cases in which they appeared, and frequency of occurrence is calculated.

14. Independent Data and Safety Monitoring Committee
  The independent data and safety monitoring committee will monitor the analyses performed as interim
analyses and judge the advisability of continuing with the trial when an adverse event has occurred.

15. Expected Outcome and Significance of the Study

At present, ways of preventing the development of food allergy through baby food are not known, and the introduction of highly allergenic foods in infants is delayed with no particular basis evidence. If it can be ascertained that early introduction of such foods decreases the development of immediate-type food allergy, it will be proven that the existing intake theory rather increases the onset of food allergies, and early intake may be recommended. This will constitute a new finding and may change the nutrient intake methods for all infants, making a huge contribution to the public and society.

16. Advantages and Disadvantages of Participation in the Study

Advantages of Participation

① Egg intake can be started safely as egg will be introduced for the first time under the supervision of a physician.
② If food allergy appears, it can be promptly treated by a medical specialist.
③ The participant will undergo regular medical checkups and receive appropriate treatment for atopic dermatitis by a medical specialist.

Disadvantages of Participation

① The participant must consume the prescribed amount of trial powder everyday at home.
② The participant cannot be given eggs or egg-containing foodstuff during the trial period.
③ Progress of the intervention must be recorded and the participant must undergo regular medical checkups once in every 1~2 months.
④ The participant must take the oral food challenge.
⑤ The participant must undergo 3 blood collections for the tests.
⑥ If the infant is already allergic to eggs at the time of enrollment in the study, there is a possibility of appearance of strong allergy symptoms, such as hives or anaphylaxis, upon consuming the trial powder.

17. Right to Participate in and Withdraw from the Study

Since the targets of the present study are infants, it is not possible to obtain their consent. Therefore, the principal investigator or the sub-investigators will obtain voluntary written consent from participants’ parents or their guardians, after adequately explaining the briefing paper containing the following explanatory items. The consent form will include the title of items explained, the full name of the subject, relationship of the legal representative for the subject, signature of the legal representative, and date of filling the form. The briefing paper will be handed over to the subject and his/her legal representative, while the consent forms will be in the custody of the study representative until completion of the present study or until 5 years have elapsed after withdrawal.

Consent can be revoked at any point of time. However, it will be impossible to remove specific individual data if the study results have been published after immobilization/analysis.
[Explanatory items]

① Objective of the study
② Study methodology
③ Study implementation period and planned duration of participation
④ The number of participants scheduled to participate in the study
⑤ Expected clinical benefits as well as disadvantages
⑥ The compensation and treatment available to the participant in the event of a study-related health hazard
⑦ That the subject’s participation in the trial is voluntary and they can revoke the consent whenever they choose. That the participant's refusal of or withdrawal from participation in the study does not cause any disadvantage to the participant.
⑧ That the guardian of the participant will be informed by the physician in a timely manner if information becomes available that may be relevant to the participant's willingness to continue participation in the study.
⑨ Withdrawal criteria for participation in the study
⑩ That the personal information of the participant will be protected while the study is being carried out and when the results are published.
⑪ The participant's responsibilities.
⑫ Full name, official title and contact address of the study representative, and contact address of the medical institution providing the consulting service.

18. Cost Burden

Cost burden of the trial

The participants will bear the cost of the hospital stay for the food challenge, charges for some of the blood tests, and charges for outpatient visits every 1~2 months, which are all covered under health insurance. For the work load on the subjects for participating in the study in the form of daily intake of trial powder and maintaining weekly records of skin condition, a remuneration of JPY 500 QUO Card per subject will be granted.

The remaining tests (IgG1, IgG4, and IgA) not covered under health insurance will be paid for through research funds and thus will not be borne by the subjects. The trial powder will be purchased using the research expenses and will be distributed to the subjects free of charge.

The food will be provided by the Kewpie Corporation, which our center has no profit sharing in. There is no load related to analyses on the participants other than tests conducted for medical examination and treatment.

Funding sources and financial relations

The present study will be implemented using MHLW research grants and grants from the NCCHD for medical research and development in all fairness, with the funding parties having no relationship with any of the researchers involved in the study.
19. Protection of Personal Information and Handling of Research Results

The registered patients will be identified or referred to using the registration numbers issued at the time of registration.

At the time of publishing the research results, the privacy of the individuals will be protected by anonymizing the research data. The research results are regarded as highly valuable as the basis for preventing onset of food allergies in infants, and will be shared with the public in the form of reports at domestic academic conferences and publications in domestic and international journals. The research results will be the intellectual property of the Division of Allergy, National Center for Child Health and Development.

20. Compensation for Health Hazards and Taking out Insurance

If any sort of damage to a participant's health results from the present study, the study representative and sub-investigators will promptly provide diagnosis and treatment within the scope of healthcare services provided by health insurance. The study representative and sub-investigators will separately take out medical professional liability insurance.

21. Regulations to be Complied with

The present study will be implemented in compliance with the final protocol, which has been created in accordance with the Helsinki Declaration and ethical principles related to clinical research.

22. Handling of Samples after Completion of the Study

Samples such as left-over serum, etc., will be kept anonymous and stored under refrigeration for a period of one year after completion of the study (expected to end in March 2017), in the custody of Department of Allergy & Immunology, National Center for Child Health and Development. If necessity to analyze these samples in a new study arises, a fresh application will be submitted to the ethics committee within this period. In all other cases, the samples will be disposed of by March 31, 2017 after being heat-sterilized.
23. Actions Related to Flow and Retraction of Samples and Information

① Samples and Flow of personal information

National Center for Child Health and Development
Treatment information data collection: Attending physician
In charge of anonymized information management: Yukihiro Ohno

Blood samples with registration no.
Test results with names

Tokushima University
The Institute for Enzyme Research
Department of Molecular Analytical Chemistry
Person in charge: Hiroshi Kido

Hospital laboratory

National Center for Child Health and Development
Research laboratory
Person in charge of sample processing/storage: Kenji Matsumoto

Subject identification table

Blood samples with registration no.
Test results with names

National Center for Child Health and Development
Personal information manager
Acting President, Planning & Strategy
President: Takashi Igarashi

Test results with registration no.
24. Application of Medical Information

As a procedure of this hospital, patient IDs are assigned to patients. Medical information such as onset of food allergy or adverse events will be used as endpoints in the present study.

25. Expected date for submitting the study plan completion report [Form 6]

March 2017

26. References

2. Bright I. Nwaru et al.: Age at the introduction of solid foods during the first year and allergic sensitization at age 5 years. Pediatrics 125, 50-59, 2011
5. Du Tois G. et al.: Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. Journal of Allergy and Clinical Immunology 122, 122, 984

7. Ikematsu Kaori et al.: [Feature of food allergy developed during infancy (1)--relationship between infantile atopic dermatitis and food allergy]. Arerugi 55, 140-150, 2006 (Japanese)

Study on the Prevention of Onset of Egg Allergy in Infants with Atopic Dermatitis

Statistical Analysis Plan

Protocol Version 0.1 (Japanese) (Created August 14, 2014)
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1 Objectives
The study targets are 4~5-month-old infants with atopic dermatitis, as they are considered to be a high-risk group for developing food allergies. After daily oral administration of heated whole egg powders to a large number of infants for 6 months (from the time they are 6 months old and start baby food until they turn 1), we will implement an oral oral food challenge once they have completed 12 months of age to investigate whether or not they have developed an egg allergy. In this way, we will investigate the efficacy of introducing egg from an early stage of baby food as a preventive therapy against the onset of immediate-type egg allergy.

2 Trial Design
2-1 Trial Design
Dual-center, double-blind, placebo-controlled, stratified, parallel-group randomized clinical trial

2-2 Trial Diagram

2-3 Subjects
The subjects are patients with atopic dermatitis who are eligible to inclusion criteria and are not eligible to exclusion criteria. The inclusion criteria and the exclusion criteria are to be referred in the trial protocol.

2-4 Trial Interventions
① Egg group
[From 6 months of age until 8 months of age]
Mixture of 50 mg of heated whole egg powder, 100 mg of pumpkin powder, and 150 mg of glucose powder
[From 9 months of age until 11 months of age]
Mixture of 250 mg of heated whole egg powder and 250 mg of glucose powder
Storage method Cryopreserved
② Placebo group
[From 6 months of age until 8 months of age]
Mixture of 150 mg of pumpkin powder and 150 mg of glucose powder
[From 9 months of age until 11 months of age]  
Mixture of 250 mg of pumpkin powder and 250 mg of glucose powder  
Storage method Cryopreserved

2-5 Sample size

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg group</td>
<td>100 infants</td>
</tr>
<tr>
<td>Placebo group</td>
<td>100 infants</td>
</tr>
<tr>
<td>Total</td>
<td>200 infants</td>
</tr>
</tbody>
</table>

Basis for Establishment

According to studies on the incidence rate of immediate-type allergy in infants with atopic dermatitis reported so far, a nation-wide prevalence study on atopic dermatitis and immediate-type food allergies, as well as the data obtained from a birth cohort study (unpublished) being implemented at this center, the incidence rate of immediate-type hen’s egg allergy in 1 year old infants with atopic dermatitis is estimated to be 7% when intervened by intake of egg, and 20% in case of placebo intake. In this study, the sample size needed to detect the difference with 80% power using a two-sided test (5%) is 92 subjects in each group. There will be 100 subjects in each group, presupposing a dropout rate of about 10%.

2-6 Study Duration

Registration Period: ~March 2016  
Follow-up Period: ~March 2017

3 Endpoints

3-1 Endpoints for efficacy

Primary Endpoint

Negative rate of diagnosis from oral food challenge implemented on 1 year old (12 months old)

Secondary Endpoint

- Serum hen’s egg-specific IgE (ImmunoCAP) at the start of the study, after 3 months, at the end.
- Serum hen’s egg and other food* specific IgG1, IgG4, and IgA (a novel allergen microarray system, which is equipped with a DLC-coated chip at the start of the study, after 3 months, at the end.  
  *other food: milk, wheat, soy, peanut, buckwheat, rice, beef, chicken, house dust, mite, dog, cat.
- Flow cytometric analysis of blood cell at the start of the study and at the end.
- Serum TARC (thymus and activation-regulated chemokine) at the start of the study, after 3 months, at the end.
- Salivary IgA, IgG1, and IgG4 measurements (DLC-coated chip) at the start of the study, after 3 months and, at the end.

3-2 Safety Endpoints
Expression of adverse events

4 Analysis Population

FAS (Full Analysis Set)
FAS consists of all randomized subjects who take at least one trial powder. Efficacy endpoints and safety endpoints are analyzed in FAS.

PPS (Per Protocol Set)
PPS consists of all randomized subjects who take trial powder and fulfill the following criteria. The analysis in PPS is secondary analysis to evaluate the efficacy endpoints.

- Fulfill all the inclusion criteria and do not meet the exclusion criteria.
- Take trial powder for at least 130 days.
- Successfully eliminated hen’s-egg containing foodstuff from the diet, except for trial powder, with accidental ingestion during trial limited to a maximum of 3 times.

5 Data Handling

- Handling of missing values
  - Regardless of the reason, missing values of the primary endpoint is handled as positive.
  - Missing values of the secondary endpoints is excluded from the analysis.
  - Handling methods for unexpected missing data is decided after discussions between trial statisticians.
- Handling of outliers
  - The outliers are treated along with the plan of data management.

6 Level of Significance

All comparisons is made using the two sided test, with level of significance per comparison being 0.05. The confidence coefficient for confidence interval is 0.95.

7 Numbers of Digits

- Frequency : Integer
- Proportion : One decimal place
- Maximum, Minimum : same decimal place as the intended variable
- Mean, Median : one less decimal point than the intended variable
- Standard deviation : two less decimal point than the intended variable
- P value : 4 decimal place

8 Software to be Used

All statistical analyses is performed using R version 3.1.0.
9  **Interim Analyses**

One interim analysis is planned in this study.

- **Timing**
  
  One year after the beginning of the study or when 100 subjects completed this study.

- **Analysis**
  
  - Point estimates for the primary endpoint for two groups are calculated. Comparison is not made based on the hypothesis test.
  
  - Conditional power is calculated using the interim results of the trial to evaluate the probability of success of the study. This evaluation is carried out by the Independent Data and Safety Monitoring Committee.
  
  - If the estimated intervention effect significantly away from the value used in the sample size calculation, the sample size is re-estimated. However, the decision regarding continuation of the trial by changing the protocol is taken by the Independent Data and Safety Monitoring Committee.

10  **Descriptive statistics**

10-1  **Protocol violations**

The number of subjects in each group, number of drop-outs, and the number of withdrawals is summarized. Also, reasons for dropping-out/withdrawal are summarized. This description is done separately for FAS and PPS.

10-2  **Status of intake of trial powder**

The number of days of the trial powder consumed is summarized.

10-3  **Summary of background information**

- For each groups of intervention and therapy for atopic dermatitis, gender, nutrition during infancy, history of allergic diseases of both parents is summarized.

- For each groups of intervention and therapy for atopic dermatitis, serum immunological test readings, POEM values, diagnosis of oral food challenge, serum TARC, etc. is summarized.

- The method for description:
  
  - For nominal data, the number of subjects and proportion (%) are used.
  
  - For continuous data, mean and standard deviation, and median, 25% value, 75% value, minimum value, and maximum value are used.

11  **Analysis of Efficacy Endpoints**

**Analysis of the Primary Endpoint**

For the primary comparison, oral food challenge results (positive/negative) of each intervention group at 12 months of age are compared using the chi-squared test. Yates continuity correction is not performed. FAS is used as the primary analysis set. Secondary analysis is made using PPS. Level of significance is set at 0.05. Point estimates of the negative test are calculated for each intervention groups and its 95% confidence
interval is obtained using the Clopper-Pearson method, which uses a binomial distribution. Similarly, point estimates of the group difference as well as its 95% confidence interval also are calculated. As secondary evaluation, analyses adjusted for severity of atopic dermatitis evaluated using SCORAD, history of allergic diseases of both parents, and baby food initiation time are conducted using the logistic regression model. In addition, the analysis using all items in the above related to the primary endpoint as adjustment factors are the sensitivity analysis of main comparison.

**Analyses of Secondary Endpoints**

- Difference of oral food challenge results (positive/negative) between proactive therapy and reactive therapy is detected by using the chi-squared test. Yates continuity correction is not performed. FAS is used as the primary analysis set. Secondary analysis is made using PPS. Level of significance is set at 0.05. Analyses adjusted for severity of atopic dermatitis evaluated using SCORAD, feeding by breast milk, and history of allergic diseases of both parents are conducted by using the logistic regression model. In addition, the analysis using all items related to the primary endpoint as adjustment factors is conducted.

- In relation to the following endpoints, Wilcoxon signed rank test is used for comparing changes within the group, and Wilcoxon rank sum test is used for comparing the group difference for the trial intervention. FAS is used as the analysis set. PPS also is used for the secondary analysis. Level of significance is set at 0.05.
  - Serum antigen and other food*-specific IgE, IgG1, IgG4, and IgA at the start of the study, after 3 months, at the end.
    
  *other food: milk, wheat, soy, peanut, buckwheat, rice, beef, chicken, house dust, mite, dog, cat, et al (total 20 items).
  - Serum TARC (thymus and activation-regulated chemokine) at the start of the study, after 3 months, at the end.
  - Salivary antigen-specific IgA, IgG1, and IgG4 at the start of the study, after 3 months, at the end.

**12 Analysis of Safety Endpoints**

Adverse events are classified into serious and not serious, the name of adverse event, number of cases in which they appeared, and frequency of occurrence is calculated. Name of adverse reaction, number of cases in which they appeared, and frequency of occurrence is calculated.
Summary of the Statistical Analysis Plan Changes

(Original Protocol (29-Feb-2012) : Protocol Version 1.0)
Amendment 1 (14-Aug-2014) : Version 0.1 (Original statistical analysis plan)
Amendment 2 (21-Aug-2012) : Version 0.2
Amendment 3 (30-Jan-2015) : Version 1.0
Amendment 4 (28-Feb-2015) : Version 1.1
Amendment 5 (15-Aug-2015) : Version 1.1 (English)
**Amendment 1 (14-Aug-2014) : Version 0.1 (Original statistical analysis plan)**

The purpose of this amendment is to:

- Make a separate Statistical Analysis Plan to describe details of statistical analysis for this trial by the statistician who joined this trial team before statistical analysis starts.
- Determine the statistical analysis method before the interim analysis starts.

The table below outlines the changes:

<table>
<thead>
<tr>
<th>Sections Changed</th>
<th>(Original Protocol Version 1.0)</th>
<th>Statistical Analysis Plan (Version 0.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 Data Handling</td>
<td>Handling of withdrawal case data</td>
<td>Handling of missing values \</td>
</tr>
<tr>
<td></td>
<td>① As to FAS, results of participant’s blood examinations and an oral food challenge test are used as outcomes even if they were carried out before the end of the trial. If those data do not exist, outcome data of the case are handled as missing values.</td>
<td>Regardless of the reason, missing values of the primary endpoint is handled as positive.</td>
</tr>
<tr>
<td></td>
<td>② As to PPS, results of participant’s blood examinations and an oral food challenge test are used as outcomes even if they were carried out before the end of the trial. If those data do not exist, outcome data of the case are handled as missing values.</td>
<td>Missing values of the secondary endpoints is excluded from the analysis.</td>
</tr>
<tr>
<td></td>
<td>③ Data fixed before the release of blinded assignment should be used as outcomes to be analyzed.</td>
<td>Handling methods for unexpected missing data is decided after discussions between trial statisticians.</td>
</tr>
<tr>
<td></td>
<td>Supplementation of missing values of oral food challenge tests. Handling after violation matters If the data of withdrawal cases are recognized as &quot;missing values&quot;, their results are handled as positive in the oral food challenge tests.</td>
<td>Handling of outliers \</td>
</tr>
<tr>
<td></td>
<td>If there are outliers that ought to be excluded from the analysis, the values as well as the reason for exclusion must be mentioned in the analysis report.</td>
<td>If there are outliers that ought to be excluded from the analysis, the values as well as the reason for exclusion must be mentioned in the analysis report.</td>
</tr>
<tr>
<td></td>
<td>The results of primary endpoint (oral food challenge) who had violation matters are treated as follows in FAS and PPS.</td>
<td></td>
</tr>
</tbody>
</table>
① Cases who take the trial powder for less than 130 days: the result of the oral food challenge test is used as primary endpoint in FAS.
② Cases who accidentally ingested egg containing foods other than the trial powder in four or more times: the actual measurement of the oral food challenge is used as primary endpoint in FAS.
③ Cases whose diary was lost or blank: the result of the oral food challenge test is used as primary endpoint in FAS and PPS.

Missing values caused by withdraw and positive results of endpoint caused by violation matter are recorded in the analytical procedure, and those are excluded in the data summary.

The other handling of cases and data not described in this protocol such as data fixation whether they should be included or excluded are determined after discussion among statistician and trial investigators. The key open is performed after data fixation.

<table>
<thead>
<tr>
<th>6</th>
<th>Level of Significance</th>
<th>(Not described)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>7</th>
<th>Numbers of Digits</th>
<th>(Not described)</th>
</tr>
</thead>
</table>

All comparisons is made using the two sided test, with level of significance per comparison being 0.05. The confidence coefficient for confidence interval is 0.95.

- Frequency: Integer
- Proportion: One decimal place
- Maximum, Minimum: same decimal place as the intended variable
- Mean, Median: one less decimal point than the intended variable
- Standard deviation: two less decimal point than the intended variable
- P value: 4 decimal place
<table>
<thead>
<tr>
<th>8 Software to be Used</th>
<th>(Not described)</th>
<th>All statistical analyses is performed using R version 3.1.0.</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 Interim Analyses</td>
<td>Difference between the two groups as to primary endpoint is checked to recalculate the sample size and assess safety of the trial.</td>
<td>Point estimates for the primary endpoint for two groups are calculated. Comparison is not made based on the hypothesis test. Conditional power is calculated using the interim results of the trial to evaluate the probability of success of the study. This evaluation is carried out by the Independent Data and Safety Monitoring Committee. If the estimated intervention effect significantly away from the value used in the sample size calculation, the sample size is re-estimated. However, the decision regarding continuation of the trial by changing the protocol is taken by the Independent Data and Safety Monitoring Committee.</td>
</tr>
<tr>
<td>11-1 Analysis of the Primary Endpoint</td>
<td>For the primary comparison, oral food challenge results (positive/negative) of each intervention group at 12 months of age are compared using the Fisher's exact test. FAS is used as the primary analysis set. Secondary analysis is made using PPS. Level of significance is set at 0.05. The 95% confidence interval of the group difference is calculated using binomial distribution.</td>
<td>For the primary comparison, oral food challenge results (positive/negative) of each intervention group at 12 months of age are compared using the chi-squared test. Yates continuity correction is not performed. FAS is used as the primary analysis set. Secondary analysis is made using PPS. Level of significance is set at 0.05. Point estimates of the negative test are calculated for each intervention group and its 95% confidence interval is obtained using the Clopper-Pearson method, which uses a binomial distribution. Similarly, point estimates of the group difference as well as its 95% confidence interval also are calculated.</td>
</tr>
</tbody>
</table>
Amendment 2 (21-Aug-2012) : Version 0.2

The purpose of this amendment is to:

- Delete one of the secondary endpoints because the flow cytometric analysis costs too much and was thought to be less essential for this trial.

The table below outlines the changes:

<table>
<thead>
<tr>
<th>Sections Changed</th>
<th>Version 0.1</th>
<th>Version 0.2</th>
</tr>
</thead>
</table>
| 3-1-2 Secondary Endpoint | ① Serum hen’s egg-specific IgE (ImmunoCAP) at the start of the study, after 3 months, at the end.  
② Serum hen’s egg and other food* specific IgG1, IgG4, and IgA (a novel allergen microarray system, which is equipped with a DLC-coated chip, as previously published (Suzuki K, Anal Chim Acta 2011; 706:321-7.)) at the start of the study, after 3 months, at the end.  
*other food: milk, wheat, soy, peanut, buckwheat, rice, beef, chicken, house dust, mite, dog, cat.  
③ Flow cytometric analysis of blood cell at the start of the study and at the end.  
④ Serum TARC (thymus and activation-regulated chemokine) at the start of the study, after 3 months, at the end.  
⑤ Salivary IgA, IgG1, and IgG4 measurements (DLC-coated chip) at the start of the study, after 3 months, at the end. | ① Serum hen’s egg-specific IgE (ImmunoCAP) at the start of the study, after 3 months, at the end.  
② Serum hen’s egg and other food* specific IgG1, IgG4, and IgA (a novel allergen microarray system, which is equipped with a DLC-coated chip, as previously published (Suzuki K, Anal Chim Acta 2011; 706:321-7.)) at the start of the study, after 3 months, at the end.  
*other food: milk, wheat, soy, peanut, buckwheat, rice, beef, chicken, house dust, mite, dog, cat.  
③ Serum TARC (thymus and activation-regulated chemokine) at the start of the study, after 3 months, at the end.  
④ Salivary IgA, IgG1, and IgG4 measurements (DLC-coated chip) at the start of the study, after 3 months, at the end. |
<p>| 5 Data Handling: Handling of outliers | The outliers are treated along with the plan of data management. | If there are outliers that ought to be excluded from the analysis, the values as well as the reason for exclusion must be mentioned in the analysis report. |
| 11-1 Analysis of the Primary | In addition, the analysis using all items in the above related to the primary endpoint as | In addition, the analysis using all items in the above related to the primary endpoint as |</p>
<table>
<thead>
<tr>
<th>Endpoint</th>
<th>adjustment factors are the sensitivity analysis of main comparison.</th>
<th>adjustment factors are confirmed the result of main comparison.</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-2 Analyses of Secondary Endpoints</td>
<td>Flow cytometric analysis of blood cell at the start of the study and at the end.</td>
<td>(Deleted)</td>
</tr>
</tbody>
</table>
Amendment 3 (30-Jan-2015) : Version 1.0

The purpose of this amendment is to:
· Confirm the statistical analysis plan before the interim analysis.

The table below outlines the changes:

<table>
<thead>
<tr>
<th>Sections Changed</th>
<th>Version 0.1</th>
<th>Version 0.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No change</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>
Amendment 4 (28-Feb-2015) : Version 1.1

The purpose of this amendment is to:

・Determine the details of statistical method before final analysis.
・Describe actually measured endpoints and delete not measured ones planned in the original protocol. Original plan was to evaluate the difference of outcomes between the patients who were treated with proactive therapy for eczema and those with reactive therapy, however, much more patients than expected desired to receive proactive therapy and many patients who started with reactive therapy was changed to proactive therapy on the way because of insufficient control of eczema. From ethical point of view, we changed our initial plan and decided to do patient’s skin therapy first resulting in giving up to compare the results of proactive therapy and reactive therapy.
・Describe accurate timing of the blood sampling to measure secondary endpoints

The table below outlines the changes:

<table>
<thead>
<tr>
<th>Sections Changed</th>
<th>Version 1.0</th>
<th>Final Statistical Analysis Plan (Version 1.1)</th>
</tr>
</thead>
</table>
| 3-1-2 Secondary Endpoint | ・Serum hen’s egg-specific IgE (ImmunoCAP) at the start of the study, after 3 months, at the end.  
・Serum hen’s egg and other food* specific IgG1, IgG4, and IgA (a novel allergen microarray system, which is equipped with a DLC-coated chip, as previously published (Suzuki K, Anal Chim Acta 2011; 706:321-7.)) at the start of the study, after 3 months, at the end.  
* other food: milk, wheat, soy, peanut, buckwheat, rice, beef, chicken, house dust, mite, dog, cat.  
・Serum TARC (thymus and activation-regulated chemokine) at the start of the study, after 3 months, at the end.  
・Salivary IgA, IgG1, and IgG4 measurements (DLC-coated chip) at the start of the study, after 3 months, at the end. | Serum antigen-specific IgE, IgG1, IgG4, and IgA at the enrollment, at 9 months of age, and at 12 months of age  
Serum TARC (thymus and activation-regulated chemokine) at the time of initial medical examination, at 9 months of age, and at 12 months of age  
Salivary antigen-specific IgA, IgG1, and IgG4 at 6, 12, and 18 months of age |
| 10-3 Summary of background information | For each groups of intervention and therapy for atopic dermatitis, gender, nutrition during infancy, history of allergic diseases of both parents is summarized.  
For each groups of intervention and therapy for atopic dermatitis, serum immunological test readings, POEM values, diagnosis of oral food challenge, serum TARC, etc. is summarized. | For each groups, gender, nutrition during infancy, history of allergic diseases of both parents, serum immunological test readings, POEM values, diagnosis of oral food challenge, serum TARC, etc. is summarized. |
### Analysis of the Primary Endpoint

As secondary evaluation, analyses adjusted for method of treatment for atopic dermatitis (proactive therapy or reactive therapy), severity of atopic dermatitis evaluated using SCORAD, feeding by breast milk, and history of allergic diseases of both parents are conducted using the logistic regression model.

### Analyses of Secondary Endpoints

#### 11-2

**Difference of oral food challenge results (positive/negative) between proactive therapy and reactive therapy** is detected by using the chi-squared test. Yates continuity correction is not performed. FAS is used as the primary analysis set. Secondary analysis is made using PPS. Level of significance is set at 0.05.

Analyses adjusted for severity of atopic dermatitis evaluated using SCORAD, feeding by breast milk, and history of allergic diseases of both parents are conducted by using the logistic regression model. In addition, the analysis using all items related to the primary endpoint as adjustment factors is conducted.

**Serum antigen and other food*-specific IgE, IgG1, IgG4, and IgA at the start of the study, after 3 months, at the end.**

*Other food: milk, wheat, soy, peanut, buckwheat, rice, beef, chicken, house dust, mite, dog, cat, et al (total 20 items).

**Serum TARC (thymus and activation-regulated chemokine) at the start of the study, after 3 months, at the end.**

**Salivary antigen-specific IgA, IgG1, and IgG4 at the start of the study, after 3 months, at the end.**

---

As secondary evaluation, analyses adjusted for severity of atopic dermatitis evaluated using SCORAD, history of allergic diseases of both parents, and baby food initiation time are conducted using the logistic regression model.

---

(Deleted)
Amendment 5 (15-Aug-2015) : Version 1.1 (English)

The purpose of this amendment is to:
- translate the protocol from Japanese to English

<table>
<thead>
<tr>
<th>Sections Changed</th>
<th>Version 1.1 (Japanese)</th>
<th>Version 1.1 (English)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>Japanese</td>
<td>English</td>
</tr>
</tbody>
</table>
Study on the Prevention of Onset of Egg Allergy in Infants with Atopic Dermatitis

Statistical Analysis Plan

Protocol Version 0.1 (Japanese) (Created August 14, 2014)
Protocol Version 0.2 (Japanese) (Created August 21, 2014)
Protocol Version 1.0 (Japanese) (Created January 30, 2015)
Protocol Version 1.1 (English) (Translated August 15, 2015)
### Amendment

<table>
<thead>
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<th>Data</th>
<th>Composer</th>
<th>Content of Amendment</th>
</tr>
</thead>
<tbody>
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<td>August 14, 2014</td>
<td>Eisuke Inoue</td>
<td>Original</td>
</tr>
<tr>
<td>0.2</td>
<td>August 21, 2014</td>
<td>Eisuke Inoue</td>
<td>The changes combined to protocol</td>
</tr>
<tr>
<td>1.0</td>
<td>February 15, 2015</td>
<td>Eisuke Inoue</td>
<td>Final version for interim analysis</td>
</tr>
<tr>
<td>1.1</td>
<td>February 28, 2015</td>
<td>Eisuke Inoue</td>
<td>Final version for final analysis</td>
</tr>
<tr>
<td>1.1</td>
<td>August 15, 2015</td>
<td>Eisuke Inoue</td>
<td>Translation from Japanese to English</td>
</tr>
</tbody>
</table>
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1 Objectives

The study targets 4~5-month-old infants with atopic dermatitis, as they are considered to be a high-risk group for developing food allergies. After daily oral administration of heated whole egg powders to a large number of infants for 6 months (from the time they are 6 months old and start baby food until they turn 1), we will implement an oral oral food challenge once they have completed 12 months of age to investigate whether or not they have developed an egg allergy. In this way, we will investigate the efficacy of introducing egg from an early stage of baby food as a preventive therapy against the onset of immediate-type egg allergy.

2 Trial Design

2-1 Trial Design

Dual-center, double-blind, placebo-controlled, stratified, parallel-group randomized clinical trial

2-2 Trial Diagram

2-3 Subjects

The subjects are patients with atopic dermatitis who are eligible to inclusion criteria and are not eligible to exclusion criteria. The inclusion criteria and the exclusion criteria are to be referred in the trial protocol.

2-4 Trial Interventions

① Egg group

[From 6 months of age until 8 months of age]
Mixture of 50 mg of heated whole egg powder, 100 mg of pumpkin powder, and 150 mg of glucose powder
[From 9 months of age until 11 months of age]
Mixture of 250 mg of heated whole egg powder and 250 mg of glucose powder
Storage method Cryopreserved

② Placebo group

[From 6 months of age until 8 months of age]
Mixture of 150 mg of pumpkin powder and 150 mg of glucose powder
[From 9 months of age until 11 months of age]
Mixture of 250 mg of pumpkin powder and 250 mg of glucose powder
Storage method Cryopreserved

2-5 Sample size
Egg group 100 infants
Placebo group 100 infants
Total 200 infants

Basis for Establishment
According to studies on the incidence rate of immediate-type allergy in infants with atopic dermatitis reported so far, a nation-wide prevalence study on atopic dermatitis and immediate-type food allergies, as well as the data obtained from a birth cohort study (unpublished) being implemented at this center, the incidence rate of immediate-type hen’s egg allergy in 1 year old infants with atopic dermatitis is estimated to be 7% when intervened by intake of egg, and 20% in case of placebo intake. In this study, the sample size needed to detect the difference with 80% power using a two-sided test (5%) is 92 subjects in each group. There will be 100 subjects in each group, presupposing a dropout rate of about 10%.

2-6 Study Duration
Registration Period: ~March 2016
Follow-up Period: ~March 2017

3 Endpoints
3-1 Endpoints for efficacy
3-1-1 Primary Endpoint
Negative rate of diagnosis from oral food challenge implemented on 1 year old (12 months old)

3-1-2 Secondary Endpoint
- Serum antigen-specific IgE, IgG1, IgG4, and IgA at the enrollment, at 9 months of age, and at 12 months of age
- Serum TARC (thymus and activation-regulated chemokine) at the time of initial medical examination, at 9 months of age, and at 12 months of age
- Salivary antigen-specific IgA, IgG1, and IgG4 at 6, 12, and 18 months of age

3-2 Safety Endpoints
Expression of adverse events

4 Analysis Population
4-1 FAS (Full Analysis Set)
FAS consists of all randomized subjects who take at least one trial powder. Efficacy endpoints and safety
endpoints are analyzed in FAS.

4-2 PPS (Per Protocol Set)

PPS consists of all randomized subjects who take trial powder and fulfill the following criteria. The analysis in PPS is secondary analysis to evaluate the efficacy endpoints.

- Fulfill all the inclusion criteria and do not meet the exclusion criteria.
- Take trial powder for at least 130 days.
- Successfully eliminated hen’s-egg containing foodstuff from the diet, except for trial powder, with accidental ingestion during trial limited to a maximum of 3 times.

5 Data Handling

- Handling of missing values
  - Regardless of the reason, missing values of the primary endpoint is handled as positive.
  - Missing values of the secondary endpoints is excluded from the analysis.
  - Handling methods for unexpected missing data is decided after discussions between trial statisticians.
- Handling of outliers
  - If there are outliers that ought to be excluded from the analysis, the values as well as the reason for exclusion must be mentioned in the analysis report.

6 Level of Significance

All comparisons is made using the two sided test, with level of significance per comparison being 0.05. The confidence coefficient for confidence interval is 0.95.

7 Numbers of Digits

- Frequency : Integer
- Proportion : One decimal place
- Maximum, Minimum : same decimal place as the intended variable
- Mean, Median : one less decimal point than the intended variable
- Standard deviation : two less decimal point than the intended variable
- P value : 4 decimal place

8 Software to be Used

All statistical analyses is performed using R version 3.1.0.

9 Interim Analyses

One interim analysis is planned in this study.

- Timing

One year after the beginning of the study or when 100 subjects completed this study.
- Analysis
  - Point estimates for the primary endpoint for two groups are calculated. Comparison is not made based on the hypothesis test.
  - Conditional power is calculated using the interim results of the trial to evaluate the probability of success of the study. This evaluation is carried out by the Independent Data and Safety Monitoring Committee.
  - If the estimated intervention effect significantly away from the value used in the sample size calculation, the sample size is re-estimated. However, the decision regarding continuation of the trial by changing the protocol is taken by the Independent Data and Safety Monitoring Committee.

10 Descriptive statistics

10-1 Protocol violations
The number of subjects in each group, number of drop-outs, and the number of withdrawals is summarized. Also, reasons for dropping-out/withdrawal are summarized. This description is done separately for FAS and PPS.

10-2 Status of intake of trial powder
The number of days of the trial powder consumed is summarized.

10-3 Summary of background information
  - For each groups, gender, nutrition during infancy, history of allergic diseases of both parents, serum immunological test readings, POEM values, diagnosis of oral food challenge, serum TARC, etc. is summarized.
  - The method for description:
    - For nominal data, the number of subjects and proportion (%) are used.
    - For continuous data, mean and standard deviation, and median, 25% value, 75% value, minimum value, and maximum value are used.

11 Analysis of Efficacy Endpoints

11-1 Analysis of the Primary Endpoint
For the primary comparison, oral food challenge results (positive/negative) of each intervention group at 12 months of age are compared using the chi-squared test. Yates continuity correction is not performed. FAS is used as the primary analysis set. Secondary analysis is made using PPS. Level of significance is set at 0.05. Point estimates of the negative test are calculated for each intervention groups and its 95% confidence interval is obtained using the Clopper-Pearson method, which uses a binomial distribution. Similarly, point estimates of the group difference as well as its 95% confidence interval also are calculated.
As secondary evaluation, analyses adjusted for severity of atopic dermatitis evaluated using SCORAD, history of allergic diseases of both parents, and baby food initiation time are conducted using the logistic regression model. In addition, the analysis using all items in the above related to the primary endpoint as
adjustment factors are confirmed the result of main comparison.

11-2 Analyses of Secondary Endpoints

In relation to the following endpoints, Wilcoxon signed rank test is used for comparing changes within the group, and Wilcoxon rank sum test is used for comparing the group difference for the trial intervention. FAS is used as the analysis set. PPS also is used for the secondary analysis. Level of significance is set at 0.05.

- Serum antigen-specific IgE, IgG1, IgG4, and IgA at the enrollment, at 9 months of age, and at 12 months of age
- Serum TARC (thymus and activation-regulated chemokine) at the time of initial medical examination, at 9 months of age, and at 12 months of age
- Salivary antigen-specific IgA, IgG1, and IgG4 at 6, 12, and 18 months of age

12 Analysis of Safety Endpoints

Adverse events are classified into serious and not serious, the name of adverse event, number of cases in which they appeared, and frequency of occurrence is calculated. Name of adverse reaction, number of cases in which they appeared, and frequency of occurrence is calculated.