Consortium for Food Allergy Research

CoFAR 12

Systems Biology of Early Atopy

Short Title: SUNBEAM

V8.0 / 30 Nov 2022

Study Sponsor(s)	The National Institute of Allergy and Infectious Diseases (NIAID)
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Protocol: CoFAR 12	Version/Date: V8.0 / 30 Nov 2022
Title: Systems Biology of Early Atopy (SUNBEAM)	
Study Sponsor: The Division of Allergy, Immunology, and Transplantation (DAIT), The National Institute of Allergy and Infectious Diseases (NIAID)	

INSTRUCTIONS:

*The original signature page must be kept for your records. Return an electronic PDF copy of the signed signature page (*as described below) to the DAIT Regulatory Management Center via the applicable DAIT RMC email address for the protocol/network:*

DAITRegulatory_CoFA@ppd.com

I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonisation (ICH) document entitled *Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2)*. Further, I will conduct the study in keeping with local legal and regulatory requirements.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

As the site Principal Investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without the written permission of the IRB and NIAID.

*[*The site Principal Investigator should sign and date at the indicated location below. A written signature/date is acceptable (e.g., scanned and sent via email as a PDF version). An electronic signature is also acceptable (e.g., sent via email as a PDF version).]*

_____

Site Principal Investigator (Print)

_____ _____

Site Principal Investigator (Signature) **Date**

Protocol Synopsis

Title	Systems Biology of Early Atopy
Short Title	SUNBEAM
Number of Sites	• 7 U.S., Consortium for Food Allergy Research (CoFAR) sites • 5 U.S., non-CoFAR sites
Study Objectives	1. To study the role and interrelationships of established and novel clinical, environmental, biological, and genetic prenatal and early-life factors in the development of allergic diseases through age 3 years, with an emphasis on food allergy and atopic dermatitis 2. To apply systems biology to identify mechanisms and biomarkers underlying the development of food allergy, atopic dermatitis, and their endotypes 3. To collect, process, and assay or store environmental and biological samples for current and future use in the study of allergic disease development
Study Design	This is a prospective cohort study in which pregnant women (at any stage of pregnancy), the offspring's biological father, and the offspring will be enrolled and the offspring will be observed from birth to age 3 years. Clinical assessments will be conducted, questionnaire information collected, and biological and environmental samples collected on the mother, father, and child in the prenatal, perinatal, and postnatal periods of the child's life.
Primary Clinical Endpoints	1. IgE-mediated, immediate-type allergy to protocol-specified foods assessed at each clinic visit, starting at the child's 5-month visit 2. Atopic dermatitis assessed at each clinic visit starting at the child's 2-month visit
Secondary Clinical Endpoints	1. Sensitization to protocol-specified foods assessed by serum IgE and skin prick testing at each clinic visit starting at the child's 5-month visit 2. Sensitization to aeroallergens assessed by serum IgE and skin prick testing at the child's 12-, 24-, and 36-month clinic visits 3. Recurrent wheeze assessed at the child's 36-month clinic visit 4. Seasonal and perennial allergic rhinitis and allergic rhinoconjunctivitis assessed at the child's 24- and 36-month clinic visits
Exploratory Clinical Endpoints	1. The resolution of allergy to individual foods, defined as the transition from allergic to non-allergic, assessed at the child's 12-, 24- and 36-month clinic visits

	2. IgE-mediated, immediate-type allergy to non-protocol-specified foods assessed at each clinic visit, starting at the child's 5-month visit 3. Non-immediate-type food allergy (specifically, food protein-induced allergic proctocolitis and food protein-induced enterocolitis syndrome) and eosinophilic esophagitis assessed at each clinic visit, starting at the child's 5-month visit
Accrual Objective	At least 2500 pregnant women and their offspring
Study Duration	Approximately 75 months: 30 months of enrollment, up to 9 months of prenatal observation, and 36 months of child observation
Inclusion Criteria	Pregnant women who meet the following criteria will be eligible: 1. Able to understand the oral and written instructions associated with study visits and procedures and provide informed consent 2. Pregnant at any stage 3. Age 18 years or older 4. Planning to give birth at a designated center 5. Agrees to enroll offspring into the study at birth 6. In the case of multiple gestation, agrees to enroll only one child who will be selected by randomized birth order Biological fathers who are able to understand the oral and written instructions associated with study visits and procedures and provide informed consent will be eligible for enrollment. The woman's enrollment is not dependent on enrollment of the biological father.
Exclusion Criteria	Pregnant women will be excluded for any of the following: 1. Inability or unwillingness to comply with study protocol 2. Serious pregnancy complication (in the judgement of the investigator) prior to enrollment 3. Fetus has a major chromosomal anomaly 4. Plans to move and would not be able to attend or bring the child to in-person visits at a study site 5. Plans to give up the child for adoption at birth 6. Pregnancy is the result of an egg donation Infants will be excluded for any of the following: 1. Delivered earlier than 34 weeks of gestation 2. Sibling already enrolled 3. Born with a significant birth defect or medical condition, and in the judgment of the investigator, participation is not in the infant's best interest

	Biological fathers who are unable or unwilling to comply with the study protocol as it pertains to their participation are not eligible for enrollment. The mother or child's enrollment is not dependent on enrollment of the biological father.
Participant Withdrawal	Participants (mother, biological father, or child) may be prematurely terminated from the study for the following reasons: 1. Consent is withdrawn 2. The participant is lost to follow-up 3. The participant dies 4. The Investigator no longer believes participation is in the best interest of the participant 5. If the child is not enrolled at birth or the child's enrollment is terminated, the mother and biological father's participation will be terminated
Participant Replacement	Withdrawn participants may be replaced during the approximate 30-month recruitment period to meet the accrual objective
Study Procedure Stopping Rules	Oral food challenges will be halted study-wide pending review by DAIT/NIAID and/or the NIAID Allergy and Asthma Data and Safety Monitoring Board (DSMB) if more than two adverse events (AEs) of Grade 4 or one Grade 5 event are attributed to this procedure. Any other study procedure will be halted study-wide pending review by DAIT/NIAID and/or the DSMB if more than one AE of Grade 4 or one Grade 5 event are attributed to that procedure.

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Table of Contents

Glossary of Abbreviations

AE	adverse event
BP	blood pressure
CFR	Code of Federal Regulations
CoFAR	Consortium for Food Allergy Research
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DSMB	Data and Safety Monitoring Board
EoE	eosinophilic esophagitis
EASI	Eczema Area and Severity Index
EDC	electronic data capture
ePRO	electronic patient-reported-outcomes
FPIAP	food protein-induced allergic proctocolitis
FPIES	food protein-induced enterocolitis syndrome
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
lgE	immunoglobulin E
IRB	Institutional Review Board
MOP	Manual of Procedures
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OB/GYN	obstetrician-gynecologist
OFC	oral food challenge
PI	Principal Investigator
SACCC	Statistical and Clinical Coordinating Center
SAE	Serious Adverse Event
SCORAD	SCORing Atopic Dermatitis
SPT	skin prick test
TEWL	transepidermal water loss

1 Background and Scientific Rationale

Food allergy affects approximately 5-10% of young children, with highest incidence in the first year of life.[1-4] Food allergy accounts for almost $25 billion per year in U.S. costs.[5] Atopic dermatitis (also known as eczema) is a major risk factor for food allergy and other allergic diseases, and atopic dermatitis has a significant intrinsic impact on child health. Atopic dermatitis affects approximately 13% of U.S. children, and approximately one-third with atopic dermatitis have moderate to severe disease.[6,7] Atopic dermatitis is a chronic disease characterized by skin barrier disruption and inflammation, with ongoing major effects on the quality of life of children and families.[6] Currently, there is no reliable way to identify those infants destined to develop atopic disease who would benefit from targeted prevention strategies. The goal of this study is to establish a birth cohort that collects prenatal and early life bio-samples and environmental samples and rigorously phenotypes young children for food allergy and AD to identify prenatal and early life markers of high risk for food allergy and atopic dermatitis, as well as biological pathways (endotypes) that result in these conditions.

Early life atopic dermatitis is a critical step in what has been conventionally termed “atopic march” and is associated with high risk of the infant developing additional allergic diseases, including food allergy (relative risk [RR] ~5-10) and asthma (RR ~3).[8,9] Early life skin barrier dysfunction and inflammation may facilitate sensitization to allergens through the skin, leading to chronic allergic inflammation.[10] There is evidence that timing of atopic dermatitis onset is critical to the development of food allergy. In one Japanese study, onset of atopic dermatitis at 1-2 months was associated with a more than 7-fold increased odds of food allergy compared to those with no atopic dermatitis, in contrast to an only approximately 2-fold increased odds with onset from 5-12 months of age.[11] In the Australian HealthNuts study, more than 15% of those with atopic dermatitis onset between 0-3 months, and 50% of those who required prescription therapies, had challenge-proven food allergy at one year.[9]

Identifying early life markers of those at risk could facilitate implementation of current prevention strategies and discovery of new prevention strategies. Current prevention strategies for food allergy, such as early food allergen introduction,[12-18] can be difficult to implement and time consuming. It would be advantageous to target such therapies to those at risk to conserve resources and promote adherence. In the absence of ways to focus studies on those at highest risk for food allergy, testing new interventions is inefficient, requiring large populations (e.g., 2000 or more) to have sufficient power to identify preventative effects. Several potential methods of prenatal and/or early life intervention to prevent eczema and/or food allergy with high potential for success have been proposed, including introduction of allergenic foods in the neonatal period, use of emollients to enhance skin barrier (e.g. NCT01142999, NCT03376243), and attempts to modify the microbiome of mother and/or infant and various dietary interventions (e.g. NCT02286999, NCT00798226). Finding biomarkers and risk factors that reliably identify a high-risk population for intervention would facilitate study of these and other approaches.

There are some markers of increased food allergy and atopic dermatitis risk. Most commonly, studies have used the presence of at least one family member with allergic disease to define a “high-risk” population, but this definition does not reliably narrow the population to truly high-risk infants, and it misses a significant proportion of those who ultimately develop food allergy. For example, the HealthNuts study found that the presence of atopic disease in one immediate family member increased the odds of food-challenge confirmed food allergy in 1-year-olds by only 40%, and that applying this criterion would exclude only 30% of infants, while missing nearly 25% of those who develop food allergy.[19] Requiring 2 or more immediate family members did increase the risk by 80%, but it would miss more than 60% of those who go on to have food allergy. Importantly, this risk was less than the risk conferred by not have siblings. While other studies, such as the SchoolNuts study of school-aged children, also in Australia, found higher risk conferred by family history, when family history is ascertained in older children there is a higher chance that recall and

ascertainment bias may affect the relationship between family history of allergy and allergic status of the index child.[20] Although it might be assumed that a family history of a specific allergic disease confers more risk, neither study found that family history of eczema and/or food allergy was consistently a stronger risk factor for food allergy than was history of allergic rhinitis.

Various biomarkers that might identify high-risk newborns have been proposed, including markers of skin barrier function, in-utero sensitization, and in-utero immune function, but so far, there are no biomarkers with enough evidence for clinical application. In the BASELINE study in Ireland, high transepidermal water loss (TEWL) at day two of life was associated with 4-times odds of food allergy at two years, but this has not yet been validated in other studies.[21] Other predictors previously identified for allergy in general include cord blood immunoglobulin E (IgE), where associations have been found between level of total cord-blood IgE and allergic outcomes, although the results are not consistent or strong, and there are little data about food allergy or eczema.[22-30] Associations between immune phenotype in cord blood and subsequent food allergy or eczema have been reported, but to-date, an assay that could be used as a biomarker to predict food allergy or eczema has not been developed.[31-48]

Recently, major technological advances in our ability to analyze immune responses and biological pathways make it possible to more rigorously search for biomarkers of these disease. “Omics” methods, including genomics, epigenomics, transcriptomics, proteomics, metabolomics, and microbiomics allow for identification of biomarkers and understanding of biological pathways on an unprecedented scale.[49] Other new technologies allow for analysis of single immune cells to understand the precise mechanisms underlying immune responses. New approaches in systems biology can integrate these data to understand allergic phenotypes.[50] Using these new methods in a prospective birth cohort with rigorous phenotyping of food allergy and eczema will allow for identification of biomarkers and biological pathways.

A prospective birth cohort will also allow for separate evaluation of the pre- and post-natal environmental factors that may contribute to the development of eczema and food allergy. It is very likely that the microbiome affects the development of eczema and food allergy;[51] however, key questions about timing and location of vulnerability, identity of protective and risk types of microbial exposures, and environmental determinants of the microbiome remain unclear. There is also some evidence that maternal diet and micronutrient exposures, skin exposures to detergents and toxins, and pollution may play a role in development of allergy, although many questions remain.

This cohort will provide samples and data to understand the determinants, natural course, and mechanisms of developing atopic disease.

2 Study Objectives

The study objectives are:

1. To study the role and interrelationships of established and novel clinical, environmental, biological, and genetic prenatal and early-life factors in the development of allergic diseases through age 3 years, with an emphasis on food allergy and atopic dermatitis.
2. To apply systems biology to identify mechanisms and biomarkers underlying the development of food allergy, atopic dermatitis, and their endotypes.
3. To collect, process, and assay or store environmental and biological samples for current and future use in the study of allergic disease development.

The study objectives are purposely stated broadly to allow for the investigation of a large number of study questions, including new questions that will arise over time. The prenatal and early-life factors that will be studied are described in Section 3.2 *Prenatal and Early-Life Risk Factors*. Most of the investigations will fall into one of the following categories:

- Identification of factors associated with the development of allergic disease in early life
- Development of statistical models that predict who will and will not develop allergic disease in early life
- Elucidation of genetic, mechanistic, and biological pathways (endotypes) that lead to allergic disease
- Identification of biomarkers that predict the development of or indicate the presence of early allergic disease

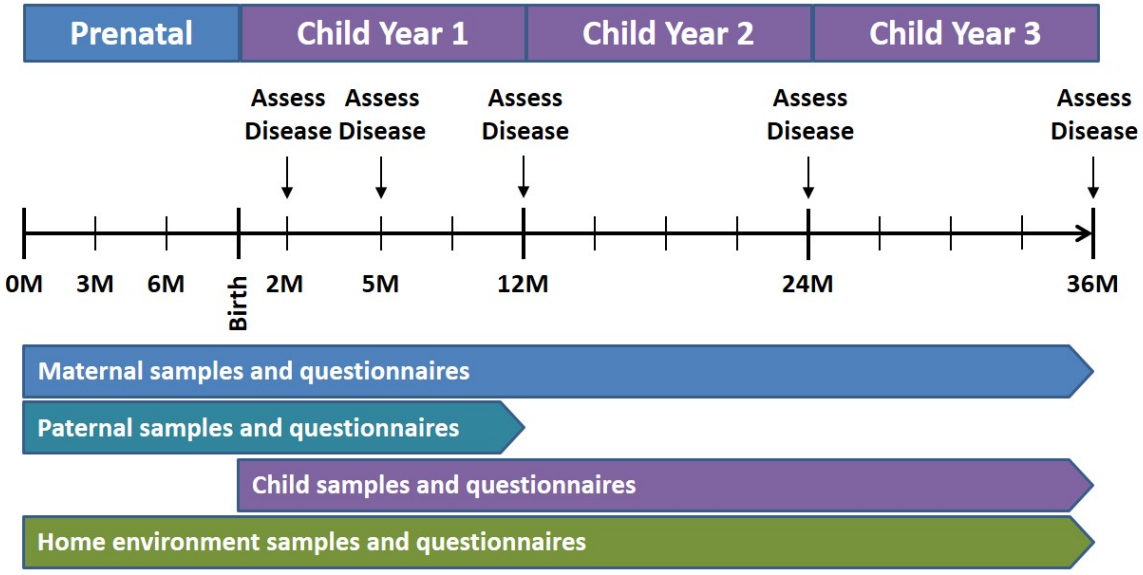
Planned mechanistic investigations and assays that have been selected for inclusion in this protocol are described in Section 7 *Planned Mechanistic Investigations and Assays*. It is anticipated that through time, additional mechanistic investigations and assays will be proposed and conducted on samples and information collected under this protocol.

3 Study Design

3.1 Description of Study Design

This is a prospective cohort study in which pregnant women (at any stage of pregnancy), the offspring's biologic father, and the offspring will be enrolled at study sites and the offspring will be observed from birth to age 3 years. The enrollment goal is at least 2500 pregnant women who agree to enroll their offspring at birth. Details of the sample size determination may be found in Section 11.8 *Sample Size Considerations*. Enrollment of biological fathers will be attempted; however, enrollment of the mother or child is not dependent on enrollment of the biological father. The enrollment period will be approximately 30 months, and the time from first-participant-first-visit to last-participant-last-visit will be approximately 75 months (30 months of enrollment + up to 9 months of prenatal observation + 36 months of child observation). During the study, biological and environmental samples and questionnaire information will be collected from the parents and the children, and the children will be assessed for allergic diseases at clinic visits at ages 2, 5, 12, 24, and 36 months (± visit windows) (Figure 3.1 *Study Overview*). The Schedules of Events (Tables 6.6A-C) provide more complete details of the study events and the timing of these events for the mother, father, and child.

Figure 3.1 Study Overview



One of the objectives of the study is to collect, process, and assay or store environmental and biological samples for current and future use. As indicated in the Schedules of Events (Tables 6.6A-C), some samples, such as urine, stool, and home dust samples, will be collected by the parent (or legal guardian; see Section 4.4 *Legal Guardians who are not the Biological Parents*) and mailed or brought to the study site, whereas other samples, such as blood and skin tapes, will be collected at the hospital at delivery and at study visits.

Because of the high cost of analyzing biological and environmental samples for the entire cohort, some samples will be collected, processed, and stored for analysis at a later date. Many questions of interest that involve associations between a disease outcome and information derived from samples can be investigated with a nested case-control design in which stored samples are analyzed only for disease cases and a random sample of controls. This design can result in substantial cost savings to the study; however, a significant limitation of the nested case-control design is that it requires the selection of a separate control group for each disease under study, and this study has multiple disease endpoints. To overcome this limitation, this study will utilize a case-cohort design that will provide a common control group across multiple case-control analyses and reduce the number of samples that have to be assayed. The design was first described in 1986 by Prentice,[52] and since then, it has become a standard design in prospective cohort studies. As pregnant women are enrolled, a subcohort of women will be randomly identified. For the analysis of a given disease, such as atopic dermatitis, all cases will be identified and selected from the entire birth cohort (inside and outside of the subcohort); however, non-cases will only be selected from inside the subcohort. Thus, the sample size for the analysis (i.e., the case-cohort sample) will be the number of participants in the subcohort (which will consist of cases and non-cases) plus all cases that arise outside of the subcohort. For the analysis, only samples collected from the subcohort and the cases that arise outside of the subcohort have to be assayed. This subcohort can be utilized for the investigation of any number of disease outcomes. Although it is anticipated that the case-cohort design will be used for most investigations involving stored samples, a nested case-control design may be utilized for a given investigation if the situation warrants.

Decisions about which study questions will be investigated with the full cohort, a case-cohort sample, or a nested case-control sample will be made throughout the life of the study and will be dependent on resources available, assay costs, suitability of samples for storage, time to complete assays, and statistical considerations. The Protocol Chairs will seek recommendations from the study investigators, the SUNBEAM Mechanistic Studies Committee, the SUNBEAM Steering Group, and the CoFAR Steering Committee about which investigations to pursue with the case-cohort sample. The SUNBEAM Steering Group is a formal committee of researchers with varying expertise that was organized to review SUNBEAM activities and to provide recommendations for study design, data and sample collections, and analyses, including mechanistic assays. Final decisions will be made by the Protocol Chairs and the NIAID in a cooperative manner.

3.2 Prenatal and Early-Life Risk Factors

Prenatal and early-life factors for investigation are shown in Table 3.2 below. These factors will be assessed through one or more of the following methods:

- Questionnaires administered by study staff or self-administered by the parent
- Medical record review
- Clinical testing
- Laboratory analysis of a collected biological or environmental sample
- Environmental exposure databases linked to the participants by geocoding

Table 3.2 is not intended to be an exhaustive list of the risk factors that might be studied under this protocol. As explained in the previous section, some biological and environmental samples will be stored for future analyses. It is anticipated that new assays will be developed and new risk factors of interest will come to light.

Table 3.2. Candidate Prenatal and Early-life Risk Factors

Prenatal and perinatal risk factors based on prior literature or strong biological plausibility	Key environmental exposures	Biologic pathways
• Fetal sex • Maternal atopic disease • Paternal atopic disease • Maternal skin barrier function • Sibling atopic disease • Birth order • Season of birth • Candidate genes • Mode of delivery • Breast feeding • Neonatal and early infancy skin barrier function • Candidate neonatal skin and stool microbiome • Cord blood IgE	• Maternal diet • Exposure to allergens in house dust • Microbial exposures in house dust • Pollution exposures from geocoding • Maternal depression and stress • Water hardness of home water • Child diet • Maternal and paternal obesity • Child and maternal vaccinations • Tobacco smoke • Maternal and child antibiotic use • Child detergent exposure • H2-receptor antagonists • Pet and farm animal exposure	• Maternal and child stool microbiome • Maternal vaginal microbiome • Maternal and child skin microbiome • Cord blood cytokines and cellular profiles • Maternal and child cytokine, cellular and antibody profiles • Genomics • Maternal and child proteomics, lipidomics, and transcriptomics • Metabolomics (urine, stool, sera) • Breast milk composition and immune factors

3.3 Primary Clinical Endpoints

3.3.1 Food Allergy

IgE-mediated, immediate-type allergy to protocol-specified foods will be assessed at each clinic visit starting at the child's 5-month visit. The foods, which are age-specific, are:

- Milk, egg, and peanut at all visits starting at the 5-month visit
- Wheat, soy, tree nuts (cashew, hazel, walnut), fish (cod), shellfish (shrimp), and sesame at the 12-, 24-, and 36-month visits.

Clinical assessments and diagnostic criteria for food allergy are described in *Section 6.4.6 Clinical Endpoint Assessments,* and the Food Allergy Algorithm for the diagnosis of food allergy and food allergy resolution can be found in Appendix A. Dependent on the study question and timepoint of interest, food allergy may be classified in a variety of ways, including, but not limited to, the following examples:

- Any food allergy versus no food allergy
- Presence or absence of allergy to a specific food, such as milk or peanut
- Number of food-specific allergies, ranging from 0-3 at age 5 months and 0-11 at older ages
- Current allergy to any food (or a specific food), past allergy to any food (or a specific food), or never had a food allergy

3.3.2 Atopic Dermatitis

Atopic dermatitis and its severity will be assessed at each clinic visit. Clinical assessments, diagnostic criteria, and severity scoring for atopic dermatitis are described in *Section 6.4.6 Clinical Endpoint Assessments*. Atopic dermatitis may be a dependent (outcome) variable for some study questions and an independent (predictor or risk factor)

variable for others. Dependent on the study question under investigation and the timepoint of interest, atopic dermatitis may be classified in a variety of ways, including, but not limited to, the following examples:

- Present versus absent
- Ever had versus never had
- Currently have, had in the past, or never had
- None, mild, moderate, or severe (or combinations of severity categories)
- Continuous or categorized SCORing Atopic Dermatitis (SCORAD) or Eczema Area and Severity Index (EASI) scores

3.4 Secondary Clinical Endpoints

3.4.1 Sensitization to Food Allergens

Sensitization to the protocol-specified foods outlined in Section 3.3.1 *Food Allergy* will be assessed by serum IgE and skin prick testing at each clinic visit starting at the child's 5-month visit. Detailed information on serum IgE and skin prick testing for both food allergens and aeroallergen can be found in the Manual of Procedures (MOP). A positive food-specific IgE test will typically be defined as an IgE concentration at or above the level of detection for the assay. A positive food-specific skin prick test (SPT) will typically be defined as a wheal ≥3 mm above the negative control wheal. However, dependent on the study question, a different threshold could be defined for either test, and some analyses could use continuous IgE and wheal size results. Sensitization could potentially be defined by IgE alone, skin test alone, either, or both. Serum IgE to egg, milk, and peanut will be also be assessed at the 2-month visit for use in mechanistic investigations but not in the determination of clinical food allergy.

3.4.2 Sensitization to Aeroallergens

Sensitization to aeroallergens will be assessed by serum IgE and skin prick testing at the child's 12-, 24-, and 36-month visits. The panel of aeroallergens will consist of the following:

- Alternaria
- Cat
- Cockroach
- Dog
- Dust mites
- Mouse
- Trees, weeds, and grass pollens (site specific)

A positive specific IgE test will typically be defined as an IgE concentration at or above the level of detection for the assay. A positive specific SPT will typically be defined as a wheal ≥3 mm above the negative control wheal. However, dependent on the study question, a different threshold could be defined for either test, and some analyzes could use continuous IgE and wheal size results. Sensitization could potentially be defined by IgE alone, skin test alone, either, or both.

3.4.3 Recurrent Wheeze

Recurrent wheeze will be assessed at the child's 36-month clinic visit and will be defined as at least two episodes of wheezing during the first three years of life, with at least one episode between the ages of 24 and 36 months. The history of wheezing episodes, other respiratory symptoms, and medication use will be assessed periodically by parental questionnaire and at each clinic visit (see *Section 6.4.6 Clinical Endpoint Assessments)*.

3.4.4 Seasonal and Perennial Allergic Rhinitis and Conjunctivitis

Seasonal and perennial allergic rhinitis and allergic rhinoconjunctivitis will be assessed at the child's 24- and 36-month clinic visits and defined according to diagnostic criteria established for the Inner-City Asthma Consortium (see the MOP). Nasal and conjunctival symptoms and medication use will be assessed periodically by parental questionnaire and at each clinic visit (see *Section 6.4.6 Clinical Endpoint Assessments)*.

3.5 Exploratory Clinical Endpoints

3.5.1 Resolution of Food Allergy

The resolution of allergy to individual foods, defined as the transition from allergic to non-allergic, will be assessed at the child's 12-, 24-, and 36-month visits. Information on the assessment of food allergy resolution is provided in *Section 6.4.6 Clinical Endpoint Assessments* and Section 17.3 *Application of the Algorithm to Food Allergy Resolution, Appendix A*.

3.5.2 Allergy to Non-protocol-specified Foods

IgE-mediated, immediate-type allergy to foods other than milk, egg, peanut, wheat, soy, tree nuts (cashew, hazel, walnut), fish (cod), shellfish (shrimp), and sesame will be assessed at each clinic visit, starting at the child's 5-month visit; however, these endpoints will not be included in the primary endpoint of immediate-type food allergy. Information on the assessment of these conditions is provided in *Section 6.4.6 Clinical Endpoint Assessments*.

3.5.3 Non-Immediate-Type Food Allergy and Eosinophilic Esophagitis

Non-immediate-type food allergy, specifically, food protein-induced allergic proctocolitis (FPIAP), food protein-induced enterocolitis syndrome (FPIES), and eosinophilic esophagitis (EoE), will be assessed at each clinic visit starting at the child's 5-month visit; however, these endpoints will not be included in the primary endpoint of immediate-type food allergy. Information on the assessment of these conditions is provided in *Section 6.4.6 Clinical Endpoint Assessments*.

4 Selection of Participants and Clinical Sites

4.1 Rationale for Study Population

The study population will include pregnant women, their offspring from birth until age 3 years, and the offspring's biological father. Although biological fathers will be recruited, their enrollment is not required. The women will be recruited from obstetrician-gynecologist (OB/GYN) and prenatal clinics and offices. Other than the eligibility criteria listed below, women will not be selected by any criteria or characteristics. The intent is to recruit a study population of children of varying risks with regards to the development of allergic diseases. The rationale is to identify risk factors in the population for the development of allergic disease. Selection of pregnant women whose offspring are at an elevated risk of allergic disease would prevent the study of the risk factors used to select the women.

4.2 Inclusion Criteria

4.2.1 Pregnant Women

Pregnant women who meet all of the following criteria are eligible for enrollment as study participants:

- Able to understand the oral and written instructions associated with study visits and procedures and provide informed consent

- Pregnant at any stage
- Age 18 years or older
- Planning to give birth at a study-site designated center
- Agrees to enroll offspring into the study at birth
- In the case of multiple gestation, agrees to enroll only one child who will be selected by randomized birth order

4.2.2 Biological Father

Biological fathers aged 18 years or older who are able to understand the oral and written instructions associated with study visits and procedures and provide informed consent will be eligible for enrollment.

4.3 Exclusion Criteria

4.3.1 Pregnant Women

Pregnant women who meet any of these criteria are not eligible for enrollment:

- Inability or unwillingness to comply with study protocol
- Serious pregnancy complication (in the judgement of the investigator) prior to enrollment
- Fetus has a major chromosomal anomaly
- Plans to move and would not be available for in-person visits at a study site
- Plans to give up her child for adoption at birth
- Pregnancy is the result of an egg donation

4.3.2 Infant

Infants who meet any of these criteria are not eligible for enrollment:

- Delivered earlier than 34 weeks of gestation
- Sibling already enrolled
- Born with a significant birth defect or medical condition, and in the judgment of the investigators, participation is not in the infant's best interest

4.3.3 Biological Father

Biological fathers who are unable or unwilling to comply with the study protocol as it pertains to the biological father's participation are not eligible for enrollment.

4.4 Legal Guardians who are not the Biological Parents

At screening for enrollment of either the mother or the child, if the biological mother intends to give the infant up for adoption, neither the mother nor the child should be enrolled (see Section 4.3 *Exclusion Criteria*); however, if the biological mother gives up legal guardianship of the child after the child is enrolled and the legal guardian wants the child to remain in the study, the child may remain enrolled as long as a legal guardian agrees to meet the child's study requirements and provides written informed consent for the child's continued participation. Throughout the protocol where it refers to the mother, father, or parent answering questionnaires about the child or collecting samples from the child and the child's primary home, the legal guardian who provides consent for the child's participation may complete those procedures.

5 Known and Potential Risks and Benefits to Participants

5.1 Risks of Study Procedures

5.1.1 Oral Food Challenges (OFCs)

Oral food challenges (OFCs) may induce an allergic response regardless of the stage of the study during which they are conducted. Allergic reactions range from mild to severe and include life-threatening reactions and death; however, the risk of a severe allergic reaction is reduced by initiating the challenge with a very small amount of the food, gradually increasing the dose over a prolonged time period, and stopping the challenge at the first sign of a reaction. Symptoms usually are short-lived (less than two hours) and may include an itchy skin rash, nausea, abdominal discomfort, vomiting and/or diarrhea, stuffy and/or runny nose, and sneezing and/or wheezing. Anaphylaxis, the most severe allergic reactions, may include severe respiratory symptoms (both laryngeal edema and bronchospasm/wheezing), circulatory symptoms (hypotension), and neurologic symptoms (altered mental status). While there have been two documented deaths of a child undergoing a food challenge in a clinical setting, this has not occurred to date during medically supervised OFCs in a research setting. If a participant has an allergic reaction during a challenge, they may need oral, IM, or IV medications. Clinic staff trained in the diagnosis and treatment of systemic allergic reactions, including a trained physician available within 60 seconds, as well as emergency medications and resuscitation equipment, will be available throughout the challenge to treat any allergic reactions.

5.1.2 Allergen Skin Prick Testing

The risks of skin prick testing are small. Skin prick testing may result in a small, pruritic hive where the test is placed. Usually, the hives resolve within 1-2 hours, although itch and swelling can occasionally last up to 24 hours. In approximately 1 out of 10,000 tests, the participant may experience systemic allergic symptoms including sneezing, ocular pruritus and tearing, rhinorrhea, and/or urticaria. Very rarely, a serious, life threatening allergic reaction may occur, but no deaths from skin prick testing using standard dosing techniques have been reported in 50 years.

5.1.3 Blood Draw

The risks associated with drawing blood include discomfort, bleeding, bruising or swelling where the needle is inserted and in rare cases, syncope or local infection. A local skin anesthetic (i.e., topical lidocaine/prilocaine cream) may be placed on the skin before the blood draw to reduce the pain of the needle. Side effects from this agent (mainly skin rash) may occur, including allergic reactions. The National Institutes of Health (NIH) guidelines for blood collection (amount and frequency based on age and weight) will be followed. Universal precautions will be followed to reduce the risk of infection.

5.1.4 Skin Swab, Stool, and Urine Sample Collections

There are no significant risks associated with skin swab, stool, and urine sample collection.

5.1.5 Transepidermal Water Loss (TEWL) Measurement

There are no known risks associated with this non-invasive skin measurement.

5.1.6 Nasal Secretion Sampling and Nasal Swab

Nasal filter paper collection and nasal swabbing may cause localized discomfort.

5.1.7 Skin Tapes

Very mild erythema may develop immediately after a series of tapes are applied on one localized area of skin, presumably due to the mild mechanical disturbance/irritation. The erythema is expected to resolve within 12 hours

without sequelae. Very rarely people may experience presyncope, nausea, or syncope. Possible bleeding and/or bruising may occur at the area. Rare and theoretical risks associated with the collection of skin tapes include the possibility of an allergic reaction to the tape or a skin infection. Since the tape is removed immediately after application, the risk of an allergic reaction is extremely low. To further mitigate the risk of an allergic reaction, skin tapes will not be collected from participants with a history of serious life-threatening reactions to tape or adhesives. The risk of skin infection is also extremely low because only superficial skin layers are removed.

5.1.8 Questionnaires

There is a possibility that participants may find questions too personal. Participants may refuse to answer any questions that make them feel uncomfortable. There is also a possibility that answers may be read by others; however, participants' information is carefully protected, so this is very unlikely.

5.2 Potential Benefits

The potential benefits for participation in the study include the following:

1. Periodic clinical assessments for atopic dermatitis, food allergy, allergic sensitization, rhinitis, conjunctivitis, and recurrent wheeze.
2. Children enrolled in this study could potentially benefit from early assessment of allergy to foods and, in some cases, where there is indeterminate evidence of food allergy, supervised introduction of peanut and other potentially allergenic foods. Current evidence that early introduction can prevent food allergy is strongest for peanut, but there is also evidence that early egg or milk introduction may help prevent egg and milk allergy, respectively, and the overall concept likely applies to other foods as well. This study will facilitate safe early introduction of foods by screening children for food allergy in infancy. In this study, we will perform physician supervised OFCs to common food allergens when the history and testing (e.g., skin testing) is otherwise insufficient to diagnose allergy or tolerance. Thus, in children who undergo food challenges, the oral challenge will either (1) establish the diagnosis of food allergy, allowing for adequate educational measures, including avoidance measures and advice about treatment of reactions, and prescription of self-administered epinephrine (i.e., EpiPen) for treatment of accidental reactions or (2) establish that food introduction is safe, both allowing for improved quality of life and providing the possibility to prevent future development of food allergy. Prior to the LEAP study, the potential for food challenge to be used as one of the tools to prevent peanut allergy was not known, but now the benefit of early introduction of peanut and other foods has been established, and thus, food challenge in infancy may provide direct medical benefits in addition to the knowledge gained. The alternative to an OFC to these foods in infancy is either home introduction, which is riskier than an OFC, or avoidance of the foods based on assumed allergy without confirmation. We now know that unnecessary avoidance of some foods is associated with an increased risk of developing clinical food allergy, and thus higher risk of allergic reactions later in life. In addition, the risk of assuming that a child is food allergic, when he/she is not actually food allergic, include the nutritional, psychological, financial and social costs of carrying a food allergy diagnosis and avoiding food.
3. Contribution of knowledge to the field of allergic disease that may facilitate future clinical testing and treatment.

6 Study Procedures

6.1 Clinical Procedures and Sample Collections

Below is a brief description of the clinical procedures and sample collections for the mother, father, and/or child. The timings of the procedures are provided in the maternal, paternal, and child's schedules of events (Section 6.6 *Visit*

Windows and Schedules of Events) and details of the procedures are provided in the Study MOPs. Participants will be notified of the results of the skin prick tests, oral food challenge results, and specific IgE results as described in their respective sections below. The remainder of studies will be for research purposes only.

Under this protocol, as stated in Study Objective 3, environmental and biological samples will be collected, processed, and assayed or stored for current or future use in studies of allergic disease development. For the sample collections listed below, anticipated assays are described; however, it is expected that new assays will be developed and new risk factors for allergic disease will come to light. Decisions about the types and timing of sample assays will fall under the collective purview of the study investigators, the SUNBEAM Mechanistic Studies Committee, the SUNBEAM Steering Group, and the CoFAR Steering Committee (see Section 3.1 *Description of Study Design*) and will be dependent, in part, on funding. Additional information on intended use of samples can be found in Section 7 *Planned Mechanistic Investigations and Assays*.

6.1.1 Addendum Guidelines Counseling

Study staff will counsel parents at the 2-month visit on the Addendum Guidelines for the Prevention of Peanut Allergy[53] and offer the option of the child receiving a Guidelines-based assessment and recommendation at any time between ages 4-6 months. The $\pm$ 1 month window for the 5-month visit provides for a Guidelines-based assessment between ages 4-6 months.

6.1.2 Allergen Skin Prick Test (SPT)

In children only, study staff will conduct allergy skin prick testing to food allergens at the 5-, 12-, 24-, and 36-month visits and to aeroallergens at the 12-, 24-, and 36-month visits. The allergens to be tested are listed in the footnotes of the Child's Schedule of Events (Table 6.6C). Caregivers will be provided with their child's allergen skin prick test results.

6.1.3 Blood

Study staff will collect blood by peripheral venipuncture from the mother at enrollment and at the child's 2-month visit, from the father at enrollment, and from the child at each clinic visit. Volumes are provided in the informed consent forms. At the child's 2-month visit, a heel stick may be conducted if venipuncture is not successful. Blood will be used for IgE analyses (see footnotes in Maternal, Paternal and Child's Schedules of Events) and studies of the immune system, genetics, and epigenetics. These IgE results will be measured in a non-CLIA-certified, research laboratory and will not be shared with participants; however, when food-specific IgE results are used because skin prick test results are not available for the clinical determination of food-specific allergy in the child at the 5-, 12-, 24-, and 36-month visits (see Food Allergy Algorithm in Appendix A), food-specific IgE will be measured in a CLIA-certified laboratory and shared with the child's parent or guardian.

6.1.4 Breast Milk

If breastfeeding, the mother will be asked to collect a breast milk sample up to 3 times during the child's first year of life. Study staff will provide the mother with home-collection kits and instructions on how to collect, store, and return the sample (samples may be collected at the child's clinic visit). Breast milk will be assayed for microbiome and immunologic and inflammatory markers.

6.1.5 Cord Blood

An umbilical cord blood sample will be collected at the hospital after delivery, processed, and shipped according to procedures outlined in the Study MOPs. Cord blood will be used in immunologic studies.

6.1.6 Hair

Study staff will collect a hair sample from the mother at enrollment and a sample from the child at each clinic visit. For each sample, strands of hair will be collected from the back of the head and as close to the scalp as possible. Hair samples will be assayed for environmental exposures that are potentially associated with allergic disease.

6.1.7 Height (or Length) and Weight

Study staff will measure the height and weight of the mother at enrollment and the 2- and 12-month clinic visits, of the father at enrollment, and of the child at each clinic visit. If the father does not come for a clinic visit, the information may be collected by questionnaire.

6.1.8 Home Dust

The mother will be asked to collect one dust sample from her home during the prenatal period and from the child's home when the child is age 2-5 months (1 sample) and age 12, 24, and 36 months. Study staff will provide the mother with collection kits and instructions on how to collect, store, and return the sample. Dust samples will be assayed for allergens and microbiome.

6.1.9 Home Water

The mother will be asked to collect one water sample from the child's home when the child is age 2-5 months (1 sample). Study staff will provide the mother with a collection kit and instructions on how to collect, store, and return the sample. Water samples will be analyzed for calcium carbonate (water hardness).

6.1.10 Nasal Secretion Sample

Study staff will collect a nasal secretion sample from the child at each clinic visit. Nasal secretions will be collected by inserting an absorbent strip (filter paper) into a nostril and then collecting the strip and fluids that have been absorbed into the strip. The procedure may be repeated in one or both nostrils. Nasal secretions will be assayed for immunologic markers.

6.1.11 Nasal Swab

Study staff will collect a nasal swab sample from the mother and father at enrollment and from the child at each clinic visit. An absorbent swab will be inserted into a nostril, rotated gently against the nasal mucosa, and then withdrawn. The procedure may be repeated in one or both nostrils. Nasal swab samples will be assayed for microbiome.

6.1.12 Oral Food Challenge (OFC)

Starting at age 5 months, allergy to protocol-specified foods will be assessed at each clinic visit. Open, graded, OFCs will be conducted when indicated by the Food Allergy Algorithm (see Appendix A). As shown in the algorithm, some combinations of food exposure history, symptomology, and SPT wheal size or specific IgE concentration require an OFC to determine the allergy status whereas other combinations do not. Large clinic-visit-windows (see Section 6.4.3 *Child's Clinic Visits and Visit Windows*) are provided to accommodate the need to conduct multiple OFCs for a given child. If an OFC is indicated, prior to the first OFC the mother or legal guardian will provide written informed consent.

6.1.13 Saliva

Study staff will collect a saliva sample from the child at each clinic visit by swabbing the inside of the child's cheeks with a saliva collection swab. Saliva will be assayed for immunologic and inflammatory markers.

6.1.14 Skin Assessment, SCORing Atopic Dermatitis (SCORAD), and Eczema Area and Severity Index (EASI)

Study staff will conduct a visual assessment of the child's skin at every clinic visit. Atopic dermatitis will be scored by SCORAD and EASI at each clinic visit.

6.1.15 Skin Swabs

Study staff will collect skin swabs from the mother at enrollment and the child's 2-month visit, from the father at enrollment, and from the child at 1-2 days after birth (or during the first 7 days at a home visit, an unscheduled study visit, or a pediatrician visit if not performed prior to hospital discharge) and then at every clinic visit. Study staff will swab an area of skin with a moistened swab near the area that will be skin taped. Skin swabs will be assayed for microbiome.

6.1.16 Skin Tapes

Study staff will collect skin tapes from the mother at enrollment and the child's 2-month visit, from the father at enrollment, and from the child at 1-2 days after birth (or during the first 7 days at a home visit, an unscheduled study visit, or a pediatrician visit if not performed prior to hospital discharge) and then at every clinic visit. A strip of sterile medical tape will be applied to an area of the skin with light pressure and then slowly removed. This procedure may be repeated on the same area of skin a maximum of 16 times. However, for the child at age 1–7 days, it may be repeated a maximum of 8 times. Skin tapes will be assayed for RNA expression, lipidomics, metabolomics, and proteomics.

6.1.17 Stool

The mother will collect a stool sample from herself during the prenatal period and when the child is 2 months of age and the father will collect a stool sample from himself at enrollment. Before the child is discharged from the hospital, study staff will collect a meconium/stool sample from the child's diaper. At the child's ages of 1-2 weeks and 1, 2, 5, 9, 12, 24, and 36 months, the mother will collect a stool sample from the child, either from the child's diaper or from a collection device placed on the toilet if the child is toilet trained. The mother and father will be provided collection kits and instructions on how to collect, store, and return the sample. Stool samples will be analyzed for the microbiome, the metabolome, and inflammatory and immunologic markers.

6.1.18 Transepidermal Water Loss (TEWL)

Study staff will measure TEWL on the mother's skin at enrollment and at the child's 2-month visit, on the father's skin at enrollment, and on the child's skin at 1-2 days after birth (or during the first 7 days at a home visit, an unscheduled study visit, or a pediatrician visit if not collected prior to hospital discharge) and at every clinic visit.

6.1.19 Urine

Study staff will collect a urine sample from the mother at enrollment and at the child's 2-month visit and from the child at each clinic visit. The child's urine will be collected from a diaper placed on the child at the visit or a urine collection device placed on the toilet if the child is toilet trained. Urine will be used to study metabolomics and inflammatory markers.

6.1.20 Vaginal Swabs

The mother will self collect a vaginal swab during the prenatal period. Study staff will provide the mother with a collection kit and instructions on how to collect, store, and return the sample. Alternatively, the vaginal swab sample can be collected during the clinic visit. Vaginal swabs will be analyzed for microbiome.

6.2 Maternal Study Procedures

6.2.1 Recruitment of Pregnant Women

Each participating study site will designate at least one labor and delivery center at which pregnant women enrolled in the study will give birth. Pregnant women will be recruited from OB/GYN and prenatal offices and clinics that see women who deliver at the designated center. Study staff will provide staff and physicians at these prenatal offices with flyers about the study and request their assistance in informing patients about the study. The flyer will contain study contact information for women who are interested in the study. At OB/GYN and prenatal offices at which physicians give permission to contact their patients, women may be recruited in person or contacted by telephone. Other methods of recruitment may include flyers posted throughout the community and television, radio, and social media advertisements, such as Google and Facebook advertisements.

Women who contact the study site by telephone will be provided information on the study and, after providing verbal permission to pre-screen, will be pre-screened over the phone for eligibility. Women who are found to be eligible by telephone pre-screening and interested in enrolling in the study will be scheduled for a Maternal Enrollment Visit at the study site.

Women recruited in person at OB/GYN and prenatal offices and clinics may be pre-screened at that time for eligibility after providing verbal permission to pre-screen. Women who are found to be eligible by pre-screening and interested in enrolling in the study will be scheduled for a Maternal Enrollment Visit at the study site. If the pre-screening site is also a study site, the enrollment visit may occur at the same visit.

6.2.2 Maternal Enrollment Visit

The procedures for this visit, as listed in the Maternal Schedule of Events (Table 6.6A), are summarized below:

- Obtain written informed consent (Remote consent with an electronic signature may be obtained: see Section 14.3 *Informed Consent Process*)
- Administer the Eligibility Checklist
- Administer questionnaires and forms (some questionnaires may be completed by the mother by electronic patient-reported-outcomes (ePRO) at the visit or at home)
- Conduct clinical procedures
- Collect biological samples
- Provide instruction on how to log into and enter information into the ePRO system and review the schedule for completing the ePRO questionnaires (see Section 6.2.7 *Maternal Self-Administered Questionnaires*)
- Provide instruction on how to collect and return her pre- and postnatal samples.
- Issue kits for the self-collected, pre- and perinatal samples (additional kits may be provided by mail or in person at study visits)
- Request that she discuss the study, at her convenience, with the biological father of her child, give the father study contact information, and ask him to contact the study staff if he is interested in participating in the study.

The woman will be enrolled upon consent and will be assigned a study identification number. The mother, father, and child will be assigned a unique study identification number that identifies the participants as individuals and as a related group.

An additional enrollment visit may be scheduled if needed to complete the enrollment tasks.

6.2.3 Reaffirmation of Child's Participation

Prior to conducting any study procedures or collecting any information on the child, study staff will ask the mother to verbally reaffirm the child's participation in the study. This verbal reaffirmation may be conducted by phone or in person at any time between 1 month before the delivery due date and the conduct of any procedures or the collection of any information on the child.

6.2.4 Maternal Study Procedures during Hospitalization for Delivery

There are no maternal questionnaires or clinical procedures to be completed on the mother during her hospitalization for delivery. If verbal reaffirmation of the child's participation has not been received, study staff should seek reaffirmation prior to collecting any samples or information on the child.

Before discharge, study staff will review the following with the mother:

- The visit window for the child's 2-month clinic visit (study staff will contact the mother to schedule the appointment)
- When and how to collect and return self-collected samples
- The schedule for completing the ePRO questionnaires

If it is not practical to review the above items during the mother's hospitalization, study staff should review the information with the mother by telephone or other means as soon as practical.

6.2.5 Maternal Clinic Visits after Delivery

The mother's clinic visits will coincide with the child's clinic visits. As shown in the Maternal Schedule of Events (Table 6.6A), the following procedures will be conducted at one or more visits:

- Administer questionnaires and forms
- Conduct clinical procedures
- Collect biological samples

Some questionnaires and sample collections scheduled for the same month as a clinic visit may be completed by the mother at home.

6.2.6 Maternal Self-Collected Samples

At the times shown in the Maternal Schedule of Events (Table 6.6A), the mother will collect vaginal, stool, breast milk, and home dust samples at home and return the samples to the study site either by mail or at the next clinic visit (vaginal and breast milk samples may be collected at the clinic visit). At the enrollment visit, the mother will be instructed on how to collect and return the samples and will be provided kits for collecting the samples. She will also be instructed on how to collect the child's samples. Instructions for these sample collections are provided in the Study MOPs.

6.2.7 Maternal Self-Administered Questionnaires

The Statistical and Clinical Coordinating Center (SACCC) will provide a web-based, password-protected, ePRO system for the mother to complete online questionnaires on a personal electronic device. The mother will complete online questionnaires at home between clinic visits and at clinic visits. Besides self-administered questionnaires that focus on her, the mother will also complete questionnaires that focus on the child (see Maternal and Child's Schedule of

Events, Tables 6.6A and 6.6C, respectively). At the enrollment visit, study staff will instruct the mother on use of the system and the schedule for completing online questionnaires.

6.3 Paternal Study Procedures

6.3.1 Recruitment of the Biological Father

Enrollment of the biological father is encouraged but not a condition of enrollment for the pregnant woman or her offspring. At the Maternal Enrollment Visit, study staff will ask the mother to contact the biological father of the expectant child at her convenience, provide him with general information about the study, and ask him to contact the study site to learn more about the study and his participation. Fathers who telephone the study site will be provided information on the study and, after providing verbal permission to pre-screen, will be pre-screened by telephone for eligibility. Fathers may also be pre-screened in person after providing verbal permission to pre-screen. Biological fathers who are interested in enrolling in the study will be scheduled for a Paternal Enrollment Visit at the study site. Biological fathers may be enrolled at any time from the prenatal period up to the child's first birthdate. Fathers who are not willing to come for a clinical visit but are willing to answer questionnaires by phone or ePRO may be enrolled.

6.3.2 Paternal In-person or Remote Enrollment Visit

For fathers who agree to come for a study visit, the procedures shown in the Paternal Schedule of Events (Table 6.6B) are summarized here:

- Administer the Eligibility Checklist
- Obtain written informed consent (Remote consent with an electronic signature may be obtained: see Section 14.3 *Informed Consent Process*)
- Administer questionnaires and forms (forms may be staff or self-administered)
- Conduct clinical procedures
- Collect biological samples
- Provide instruction on how to collect, store, and return the self-collected stool sample
- Issue kit for the self-collected stool sample

The father will be enrolled upon consent and assigned a study identification number. An additional enrollment visit may be conducted if required, and some questionnaires and the stool sample collection may be completed at home.

If the father agrees to participate but is not willing to come for a clinic visit, the father may be enrolled by telephone. The study staff will consent the father verbally and administer the questionnaires listed in the Paternal Schedule of Events (Table 6.6B) by telephone during that call or on one or more additional calls or allow the father to complete questionnaires by ePRO. Clinical procedures will not be performed and samples will not be collected from fathers who enroll but do not wish to attend a clinic visit.

6.4 Child's Study Procedures

6.4.1 Child's Screening and Enrollment

Prior to conducting any study procedures or collecting any information on the child, study staff will ask the mother to verbally reaffirm the child's participation in the study. If the mother does not want the child to participate, the consent the mother signed at enrollment will be withdrawn and the child will not be enrolled. Once verbal

reaffirmation of consent is obtained, study staff will screen the child for eligibility by reviewing the medical record and interviewing the mother. If the child is eligible, the study staff will assign the child a study identification number. If the child is not eligible, the mother's and father's enrollment will be terminated; however, maternal and paternal study information and samples will be retained. The mother may withdraw consent for herself and her child at any time.

6.4.2 Child's Study Procedures at Birth and before Hospital Discharge

Prior to the child's discharge, the study staff will conduct the procedures and collect the biologic samples listed in the Child's Schedule of Events (Table 6.6C). If study staff are unable to conduct the procedures or collect the samples prior to discharge, the staff may perform the procedures within 7 days at a home visit, an unscheduled study visit, or a pediatrician visit.

6.4.3 Child's Clinic Visits and Visit Windows

The child will have clinic visits at age 2, 5, 12, 24, and 36 months. The 2-, 5-, and 12-month visits have a window of $\pm$ 1 month during which one or more visits may occur dependent on the child's requirements for testing. The 5- and 12-month window may be extended to +2 months for children who require an OFC to multiple foods. The 24- and 36-month visits have a window of $\pm$ 3 months (with no further extension for additional testing) during which one or more visits may occur dependent on the child's requirements for testing.

As shown in the Child's Schedule of Events (Table 6.6C), the following procedures will be conducted at each visit:

- Administer questionnaires and forms to the mother (forms may be staff or self-administered)
- Collect biological samples
- Conduct clinical procedures and assessments

6.4.4 Child's Samples Collected at Home

At timepoints shown in the Child's Schedule of Events (Table 6.6C), a parent will collect samples from the child and the child's primary home defined as the home at which the child sleeps most nights. The parent will return the samples to the study site either by mail or at the next clinic visit. Study staff will instruct the parents on sample collection procedures and provide them with sample collection kits. Details of sample collection procedures are provided in the Study MOPs.

6.4.5 Administration of Child Questionnaires

At study visits, parents will answer staff-administered and self-administered questionnaires that ask for information about the child. Self-administered questionnaires will be accessed through the online ePRO system described in Section 6.2.7 *Maternal Self-administered Questionnaires*. Between visits, parents will be asked to complete periodic questionnaires through the ePRO system.

6.4.6 Clinical Endpoint Assessments

6.4.6.1 <u>IgE-mediated, immediate-type Food Allergy</u>

Information on exposure to foods and symptoms of food allergy will be collected periodically by questionnaire starting in early infancy, and IgE-mediated, immediate-type allergy to individual, protocol-specified foods will be assessed at each clinic visit starting at age 5 months. At the 5-month visit, the clinical assessment and diagnosis of food allergy will be limited to milk, egg, and peanut. At the 12-, 24, and 36-month visits, the clinical assessment and diagnosis of food allergy will be limited to milk, egg, peanut,

wheat, soy, tree nuts (cashew, hazel, walnut), fish (cod), shellfish (shrimp), and sesame. The classification of a child as allergic to a specific food will be based on the Food Allergy Algorithm (see Appendix A) that uses history of food exposure and reactions or tolerance, IgE and SPT results, and, when recommended by the algorithm, an OFC result. Baked forms of milk and egg will not be evaluated by the algorithm.

Information on the resolution of food allergy will be assessed periodically by questionnaire and assessed at the 12-, 24-, and 36-month visits by the same Food Allergy Algorithm used for the diagnosis of food allergy (Appendix A); however, special rules for the application of the Food Allergy Algorithm to food allergy resolution will apply (see Section 17.3 *Application of the Algorithm to Food Allergy Resolution, Appendix A*).

Information on IgE-mediated, immediate-type allergy to foods not on the protocol-specified list will be collected periodically by questionnaire starting in early infancy and assessed at each clinic visit starting at age 5 months; however, the diagnosis of allergy to these additional foods will not be determined by the Food Allergy Algorithm or by study-conducted OFCs. Allergy to a non-protocol-specified food will not be included in the primary endpoint for food allergy.

6.4.6.2 Atopic Dermatitis

Atopic dermatitis is defined as the following since the last assessment (or since birth for the 2- and 5-month visits)

1. A history of a dry or itchy rash that is (a) either continuous or intermittent lasting at least 4 weeks OR (b) requiring medicated treatment AND

2. The rash was or is present in the skin creases (folds of elbows, behind the knees, fronts of ankles, or around the neck) or on the extensor aspects of the forearms or lower legs or on cheeks or trunk.

Any infant fulfilling these criteria but who, on examination by a suitably trained health professional, is deemed to have a different skin disease that explains the above findings will be classified as not having atopic dermatitis.

6.4.6.3 Sensitization to Food Allergens

Sensitization to food allergens will be assessed by serum IgE testing and skin prick testing at the 5-, 12-, 24-, and 36-month visits. For IgE testing at any given timepoint, a positive food-specific test will be defined as an IgE concentration at or above the level of detection for the assay. For skin prick testing at any given timepoint, a positive food-specific test will be defined as a wheal ≥3 mm above the negative control wheal. For either test, different thresholds could be defined for specific analyses, and sensitization could potentially be defined by IgE alone, skin test alone, either, or both. Details of the procedures are provided in the Study MOPs. Serum IgE to egg, milk, and peanut will be also be assessed at the 2-month visit, but for use in mechanistic investigations and not for the determination of clinical food allergy.

6.4.6.4 Sensitization to Aeroallergens

Sensitization to aeroallergens will be assessed by serum IgE and skin prick testing at the 12-, 24-, and 36-month visits. For IgE testing at any given timepoint, a positive specific test will be defined as an IgE concentration at or above the level of detection for the assay. For skin prick testing at any given timepoint, a positive specific test will be defined as a wheal ≥3 mm above the negative control wheal. For either test,

different thresholds could be defined for specific analyses, and sensitization could potentially be defined by IgE alone, skin test alone, either, or both. Details of the procedures are provided in the Study MOPs.

6.4.6.5 Recurrent Wheeze

Information on wheezing episodes, other respiratory symptoms, and medication use will be collected periodically by questionnaire starting in early infancy and assessed at each clinic visit for the interval of time since the previous visit (since birth for the 2-month visit) (see Child's Schedule of Events Table 6.6C). Recurrent wheeze, assessed at age 3 years, will be defined as at least two episodes of wheezing during the first three years of life, with at least one episode between the ages of 24 and 36 months.

6.4.6.6 Seasonal and Perennial Allergic Rhinitis and Conjunctivitis

Information on nasal and conjunctival signs, symptoms, and medication use will be collected periodically by questionnaire starting in early infancy and assessed at each clinic visit for the interval of time since the previous visit (since birth for the 2-month visit). At age 36 months, a determination will be made of seasonal or perennial allergic rhinitis and allergic rhinoconjunctivitis according to diagnostic criteria provided in the MOP.

6.4.6.7 Non-Immediate-Type Food Allergy and Eosinophilic Esophagitis

Information on non-immediate-type food allergy (specifically, FPIAP and FPIES)[54] and EoE will be collected periodically by questionnaire starting in early infancy and assessed at each clinic visit starting at age 5 month. These endpoints will not be included in the primary endpoint for food allergy.

FPIAP typically presents in infants who are generally healthy but have visible specks or streaks of blood mixed with mucous in the stool.[54] Food-specific IgE is typically undetectable. The diagnosis is typically clinical (improvement in symptoms with dietary exclusion), and a lack of systemic symptoms, vomiting, diarrhea and poor growth differentiate this disorder from other gastrointestinal disorders that may have blood in the stool. For this study, the diagnosis will be based on parental report fulfilling the above clinical characteristics captured by parental questionnaire. We will also seek parental history of physician-confirmed blood in the stool (by visual confirmation or guaiac testing).

FPIES is a non–IgE-mediated food allergy that typically presents in infancy, with repetitive protracted vomiting that begins approximately 1 to 4 hours following ingestion of the trigger food ("acute" FPIES reaction). Vomiting may be accompanied by lethargy and pallor and diarrhea may follow. The delayed onset of symptoms and absence of skin or respiratory symptoms suggest a systemic reaction that is distinct from IgE-mediated food anaphylaxis. Severe reactions can progress to hypothermia, methemoglobinemia, acidemia, and hypotension, mimicking sepsis. A "chronic" form of FPIES may occur when the offending food is ingested on a regular basis. The diagnosis is primarily based on the clinical history of typical characteristic signs and symptoms with improvement after withdrawal of the suspected trigger food. Specific diagnostic criteria have been proposed[55] and are included in Appendix B. For the purposes of this study, FPIES will be recorded when the diagnosis fulfills the criteria in Appendix B, based on parental report.

EoE is a chronic inflammatory disorder characterized by eosinophilic inflammation of the esophagus resulting in esophageal dysfunction.[56] The index of suspicion is increased for children with atopic comorbidities such as asthma and atopic dermatitis, food allergy, and in those with a family history of EoE. An esophageal biopsy is generally undertaken when there is a suggestive history of esophageal dysfunction

(dysphagia, food impaction, food refusal, failure to progress with food introduction, heartburn, regurgitation, vomiting, chest pain, odynophagia, abdominal pain, and malnutrition). The diagnosis requires the demonstration of esophageal eosinophilia (>=15 eos/hpf [~60 eos/mm2]). If the biopsy is positive for eosinophilia, non-EoE disorders should be excluded. For this study, evaluations for EoE will not be required or undertaken as part of the study, but the diagnosis will be recorded if there is a history of a physician diagnosis of EoE and a positive biopsy result obtained during clinical care outside of the study.

6.5 Unscheduled and Out-of-Window Visits

Procedures or sample collections scheduled for the child's first 2 days of life can be performed within 7 days at a home visit, an unscheduled study visit, or a pediatrician visit. None of these visits will be considered a protocol deviation.

If parental or investigator concerns arise between regularly scheduled visits, such as for an adverse event (AE) following a study procedure, parents may be asked to return to the study site for an unscheduled visit.

If a child misses a regularly scheduled visit and has missed the window for the visit, every attempt should be made by the study team to bring the child in for an unscheduled visit at which all of the procedures for the missed visit would be conducted. However, if the date being considered for an unscheduled visit is closer to the next scheduled visit than the missed visit, an unscheduled visit should not be conducted and the child should be brought in on the date of the next scheduled visit. An out-of-window, unscheduled visit will be considered a protocol deviation.

6.6 Visit Windows and Schedules of Events

Study visits should take place within the time limits specified in the Schedules of Events shown below in Tables 6.6A-C.

Table 6.6A. Maternal Schedule of Events

Maternal Events	Prenatal			Year 1														Year 2												Year 3											
	Pre-screen[a]	Enroll Visit[b]	Prenatal	Birth	1-2 d	1-2 w	1 m	2 m Visit(s)[c]	3 m	4 m	5 m Visit(s)[c]	6 m	7 m	8 m	9 m	10 m	11 m	12 m Visit(s)[c]	13 m	14 m	15 m	16 m	17 m	18 m	19 m	20 m	21 m	22 m	23 m	24 m Visit(s)[c]	25 m	26 m	27 m	28 m	29 m	30 m	31 m	32 m	33 m	34 m	35 m	36 m Visit(s)[c]
Maternal Questionnaires and Forms																																									
Pre-screening verbal consent and questionnaire	X																																							
Written informed consent		X																																						
Eligibility and enrollment checklist		X																																						
Contact information	X	X						X			X							X											X											X
Sociodemographics (multiple forms)		X																																						
Medical history of biological family		X						X			X							X																							
Pre- and postnatal medications and vaccinations								X																																	
Allergic and respiratory diseases		X																X																							
Maternal Smoking		X						X			X							X																							
Maternal exposure to secondhand smoke								X																																	
Maternal household chemical exposures								X																																	
Perceived stress scale		X						X			X							X											X											X
Depression scale		X						X			X							X											X											X
Diet questionnaire (DHQ3)		X																																						
Diet questionnaire (ASA24)								X																																	
Maternal allergenic foods		X					X				X																														
Maternal Clincial Procedures																																									
Transepidermal water loss (TEWL)		X						X																																	
Height and weight measurements		X						X										X																							
Maternal Sample Collections																																									
Blood		X						X																																	
Skin tapes		X						X																																	
Skin swabs		X						X																																	
Vaginal swab		X																																							
Urine		X						X																																	
Nasal swab		X																																							
Hair		X																																							
Stool			X					X																																	
Breast milk								X			X							X																							
Home dust collection			X																																						
Maternal Clinical Laboratory Tests																																									
Total and specific IgE		X[d]																																							

[a] May take place in person or by telephone

[b] Some questionnaires listed for the enrollment visit may be completed at home. An additional enrollment visit may be conducted if required.

[c] The window for the 2-, 5-, and 12-month visits is ± 1 month and the window for the 24- and 36-month visits is ± 3 months. Assessments at each of these age-based visits may require multiple visits; however, these visits should occur within the visit window. The 5- and 12-month windows may be extended to +2 months if the child requires testing for multiple foods. Some questionnaires and sample collections scheduled for the month of a clinic visit may be completed at home.

[d] Milk, egg, peanut, wheat, soy, tree nuts (cashew, hazel, walnut), fish (cod), shellfish (shrimp), and sesame

Table 6.6B. Paternal Schedule of Events

Paternal Events	Pre-Screen[a,b]	Enroll Visit[a,b]
Paternal Questionnaires and Forms		
Prescreen verbal consent and questionnaire	X	
Written or verbal informed consent		X
Eligibility and enrollment checklist		X
Contact information	X	X[c]
Sociodemographics (multiple forms)		X
Medical history		X
Allergic and respiratory diseases		X
Paternal smoking		X
Perceived stress scale		X
Depression scale		X
Paternal Clinical Procedures		
Transepidermal water loss		X
Height and weight measurements		X
Paternal Sample Collections		
Blood		X
Skin tapes		X
Skin swab		X
Nasal swab		X
Stool		X
Paternal Clinical Laboratory Tests		
Total and specific IgE		X[d]

[a] The biological father's pre-screening and enrollment visit may be completed within the child's first year of life. An additional enrollment visit may be conducted if required. Some questionnaires and the stool sample collection may be completed at home.

[b] Pre-screening and enrollment may take place in person or by telephone. If the father refuses an in-person enrollment visit, enrollment, including consent, may be conducted by telephone. In these cases, clinical procedures will not be performed and samples will not be collected; however, to be enrolled, the father must agree to complete questionnaires by phone or ePRO.

[c] The father's contact information should be updated at least annually

[d] Milk, egg, peanut, wheat, soy, tree nuts (cashew, hazel, walnut), fish (cod), shellfish (shrimp), and sesame

Table 6.6C. Child's Schedule of Events

	Prenatal			Year 1														Year 2												Year 3											
Child's Events	**Pre-screen**	**Enroll Visit**	**Prenatal**	**Birth**	**1-2 d**	**1-2 w**	**1 m**	**2 Visit(s)[b]**	**3 m**	**4 m**	**5 m Visit(s)[b]**	**6 m**	**7 m**	**8 m**	**9 m**	**10 m**	**11 m**	**12 m Visit(s)[b]**	**13 m**	**14 m**	**15 m**	**16 m**	**17 m**	**18 m**	**19 m**	**20 m**	**21 m**	**22 m**	**23 m**	**24 m Visit(s)[b]**	**25 m**	**26 m**	**27 m**	**28 m**	**29 m**	**30 m**	**31 m**	**32 m**	**33 m**	**34 m**	**35 m**	**36 m Visit(s)[b]**
Child's Questionnaires and Forms																																									
Reaffirm child's participation with mother			X	or X																																				
Eligibility and enrollment checklist				X																																				
Birth record abstraction				X																																				
Sociodemographics (multiple forms)								X			X							X											X												X
Medical history								X			X				X			X			X			X			X			X			X			X			X		X
Allergic and respiratory diseases							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Child's exposure to secondhand smoke								X			X							X											X												X
Child's household chemical exposures								X			X							X											X												X
Allergenic foods and symptoms							X	X	X	X	X	X	X	X	X	X	X	X			X			X			X			X			X			X			X			X
Infant Feeding Practices							X		X		X		X		X		X	X																								
Block Food Questionnaire: Kids 2-7 Years																														X											X
Household consumption of allergenic food									X																																	
Oral food challenge consent											X[c]							X[c]												X[c]												X[c]
Child's Clinical Procedures																																									
Skin assessment								X			X							X											X												X
SCORAD and EASI								X			X							X											X												X
Transepidermal water loss					X[a]			X			X							X											X												X
Height (length) and weight measurements								X			X							X											X												X
Addendum Guidelines counseling								X																																		
Allergen skin prick test											X[d]							X[e,f]												X[e,f]												X[e,f]
Oral food challenge when indicated											X							X											X												X
Child's Sample Collections																																									
Cord blood				X																																					
Blood								X[g]			X							X											X												X
Skin tapes					X[a]			X			X							X											X												X
Skin swabs					X[a]			X			X							X											X												X
Urine								X			X							X											X												X
Saliva								X			X							X											X												X
Nasal secretion sample								X			X							X											X												X
Nasal swab								X			X							X											X												X
Hair								X			X							X											X												X
Meconium or Stool					X	X	X	X			X				X			X											X												X
Child's home dust collection									X									X											X												X
Child's home water sampling									X																																	
Child's Clinical Laboratory Tests																																									
Specific IgE								X[d,h]			X[d]							X[e,f]												X[e,f]												X[e,f]

[a] Assessments and sample collections should occur before hospital discharge if possible, but may be performed within 7 days at a home visit, an unscheduled study visit, or a pediatrician visit (does not apply to the 1-2 day meconium/stool sample)

[b] The window for the 2-, 5-, and 12-month visits is ± 1 month and the window for the 24- and 36-month visits is ± 3 months. Assessments at each of these age-based visits may require multiple visits; however, these visits should occur within the visit window. The 5- and 12-month windows may be extended to +2 months if the child requires testing for multiple foods. Some questionnaires and sample collections scheduled for the month of a clinic visit may be completed at home.

[c] The oral food challenge consent is administered only once prior to an indicated oral food challenge

[d] Milk, egg, and peanut

[e] Milk, egg, peanut, wheat, soy, tree nuts (cashew, hazel, walnut), fish (cod), shellfish (shrimp), and sesame

[f] Alternaria, cat, cockroach, dog, dust mite, mouse, and site-specific tree and grass pollens

[g] Venipuncture or heel stick if venipuncture is unsuccessful

[h] For mechanistic investigations only, not for clinical use in diagnosing food allergy

7 Planned Mechanistic Investigations and Assays

Planned mechanistic investigations and assays revolve around the central hypothesis that the development of food allergy and atopic dermatitis results from dysregulation of barrier function, myeloid cell function, lymphoid cell function, microbiome, additional molecular influences, and importantly, interactions between them. The planned mechanistic assays and biospecimen collections will also enable study of respiratory allergy as a secondary outcome.

In addition to collecting data and samples from subjects over multiple time points during this cohort study to address the following mechanistic study areas and hypotheses, we will also bank samples from all subjects throughout the study to enable additional research that may be beyond the scope of the study areas outlined and/or are as yet unanticipated given the current state of science.

Analyses consistent with the case cohort design for the overall study will be used. An overarching goal will be to perform the following studies on a maximally overlapping sample to enable systems-based analyses across the mechanistic study areas.

7.1 Barrier Function Studies

These studies will test the hypothesis that the development of atopic dermatitis and food allergy results from dysregulated skin barrier. Barrier function studies will include measurements of TEWL and skin tape analyses for proteins, lipids, RNA transcripts/transcriptome, and skin microbiome. These studies will assess non-lesional, non-flexural skin. When possible, lesional non-flexural skin may also be assessed.

7.2 Peripheral Blood Phenotype and Function Studies

These studies will test the hypothesis that the development atopic dermatitis and food allergy results from dysregulated leukocyte function. Phenotypic analysis of whole cord and peripheral blood at steady state will be performed using flow cytometry to study the composition of peripheral blood across early life. Unsupervised clustering of cell populations based on surface marker expression will be used to identify novel cellular phenotypes that differ between clinical groups. Phenotypic analysis of stimulated peripheral blood mononuclear cells may also be performed. Biological samples permitting, findings will be further explored to (1) determine the cell source of cytokine by intracellular cytokine staining, and (2) determine the stability of the phenotype through early life.

7.3 Lymphoid Cell Function Studies

These studies will test the hypothesis that the development of atopic dermatitis and food allergy results from dysregulated lymphoid cell function. Across early lifetime points, T cell and B cell phenotypic analysis of cord and peripheral blood from a selected subset of the birth cohort will be undertaken to assess diversification of adaptive immune specific response as it relates to phenotypic polarization. Assays under consideration include total B cell receptor and total T cell receptor analysis, allergen-specific B cell and T cell sorting and transcriptome sequencing.

7.4 Microbiome Studies

These studies will test the hypothesis that the development of atopic dermatitis and food allergy results from dysregulated microbiome. Nasal, stool, skin, vaginal, dust, and breast milk microbiomes will be profiled using 16s rRNA sequencing and/or shotgun metagenomic sequencing of DNA.

7.5 Additional Molecular Influences

Data that will complement the above studies and also serve as a core dataset for discovery themselves, will be generated. These may include genomics, whole blood transcriptome, plasma metabolomics, stool and urine

metabolomics, plasma proteomics, and blood-based epigenomic profiles from participants over the study time points. Additionally, total serum IgE, specific IgE and possibly IgG_4 levels to allergens will be measured over time. These allergens may include (a) food allergens including milk, egg, peanut, wheat, soy, tree nuts, fish, shellfish, sesame and (b) aero-allergens including Alternaria, cat, cockroach, dog, dust mites, mouse, trees, weeds, and grass pollens.

7.6 Respiratory Studies

These studies will test the hypothesis that dysregulation of airway inflammatory pathways in early life contributes to the development of rhinitis, conjunctivitis and recurrent wheezing. Nasal swabs and fluid will be collected over the duration of the study to serve as a biospecimen resource for examining nasal microbiome/metagenomics and inflammatory markers.

7.7 Systems Biology

An objective of this study is to use systems biology analytic approaches to test the hypothesis that the development of food allergy and atopic dermatitis results from interactions between dysregulated barrier function, leukocyte function, lymphoid cell function, microbiome, and additional molecular influences and to identify risk signatures and molecular pathways associated with these conditions. Building on the aggregate data generated from the above assays, ideally on a maximally overlapping sample where each participant has been profiled by multiple molecular dimensions, we will perform systems-based analyses to characterize interactions and networks between the molecular inputs that lead to the development of food allergy and atopic dermatitis. Methods will be developed and applied that may include machine learning, Bayesian analysis, information theory, and additional strategies relevant at the time of analysis.

8 Biospecimen Storage

All biological samples will be stored short-term at the study site prior to shipment to facilities to be determined for storage or to laboratories for analyses. For studies requiring analyses of stored samples, the SUNBEAM storage facilities will send selected samples to laboratories for analyses.

9 Criteria for Participant and Study Completion and Premature Study Termination

9.1 Participant Completion

Participation of the mother, father, and child will be complete upon of the child's 36-month clinic visit.

9.2 Participant Stopping Rules and Withdrawal Criteria

Participants (mother, child, or biological father) may be prematurely terminated from the study for the following reasons:

1. Consent is withdrawn
2. The participant is lost to follow-up
3. The participant dies
4. The site investigator no longer believes participation is in the best interest of the participant
5. If the child is not enrolled at birth or the child's enrollment is terminated, the mother's and biological father's participation will be terminated

9.3 Participant Replacement

Withdrawn participants may be replaced at any time during the study recruitment period to achieve the accrual objective.

9.4 Study Procedure Stopping Rules

Oral food challenges will be halted study-wide pending review by DAIT and/or the NIAID Allergy and Asthma Data and Safety Monitoring Board (DSMB) if more than two AEs of Grade 4 or one Grade 5 event are attributed to this procedure. Any other study procedure will be halted study-wide pending review by DAIT and/or the DSMB if more than one AE of Grade 4 or one Grade 5 event are attributed to that procedure.

10 Safety Monitoring and Reporting

10.1 Overview

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting those data. AEs that are classified as serious according to the definition of health authorities will be reported promptly to DAIT/NIAID. Appropriate notifications will also be made to all site principal investigators (PIs), Institutional Review Boards (IRBs), and the DSMB.

In this observational study, only AEs attributable to a study procedure will be entered into the study database and reported to DAIT/NIAID and the oversight boards. Criteria for determining the relatedness of an AE to a study procedure and grading its severity are provided in Sections 10.3.1 *Grading Criteria* and 10.3.2 *Attribution Definitions*. As explained further in Section 10.3.1, AEs coded as an allergic reaction will be graded according to different criteria than non-allergic reactions.

10.2 Definitions

10.2.1 Adverse Event

For this observational study, an AE is defined as any untoward or unfavorable medical occurrence in a participant that is associated with a study procedure mandated by the protocol. This definition excludes all untoward or unfavorable medical occurrences not related to a study procedure, including hospitalizations or deaths. The study mandated procedures are listed in the Schedules of Events (Tables 6.6A-C).

10.2.2 Unexpected Adverse Event

An AE is considered “unexpected” if it is not listed at the specificity, severity or rate of occurrence that has been observed or is not consistent with the risk information described in this protocol.

10.2.3 Serious Adverse Event (SAE)

An AE is considered “serious” if, in the view of either the investigator or the DAIT Medical Monitor, it results in any of the following outcomes:

- Death.
- A life-threatening event: An AE is considered “life-threatening” if, in the view of either the investigator or the DAIT Medical Monitor, its occurrence places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization.

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Congenital anomaly or birth defect.
- Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Only Serious Adverse Events (SAEs) related to a study procedure will be entered into the study database and reported.

10.3 Grading and Attribution of Adverse Events

10.3.1 Grading Criteria

10.3.1.1 Adverse Events Not Coded as an Allergic Reaction

AEs not coded as an allergic reaction will be graded on a scale of 1-5 according to the criteria set forth in Table 10.3.1.1A (Grade 5, Death, is omitted from the table). AEs not coded as an allergic reaction but not described in Table 10.3.1.1A will be graded on a scale of 1-5 according to the criteria set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0, as shown in Table 10.3.1.1B.

Table 10.3.1.1A. SUNBEAM Grading Criteria for Adverse Events Not Coded as an Allergic Reaction

Dermatologic	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4
Bruising	2 - 5 cm	>5 cm	NA	NA
Change in Skin Color (within 2 weeks)	Slight or localized change in skin color/pigmentation	Marked or generalized change in skin color/pigmentation	NA	NA
Cellulitis	NA	Non-parenteral treatment required (e.g. oral antibiotics, antifungals, antivirals)	IV treatment required (e.g. IV antibiotics, antifungals, antivirals)	Life-threatening consequences (e.g. sepsis, tissue necrosis)
Rash (not urticarial)	No medication required	Treated with topical medications	Treated with oral medication	Hospitalization required
Erythema/Redness	Localized, lasting longer than 24 hours	Localized, lasting longer than 48 hours	Diffuse, or lasting longer than 72 hours	Necrosis or exfoliative dermatitis
Induration/Swelling	Localized and does not interfere with activity	Diffuse or interferes with activity	Diffuse and prevents daily activity	Necrosis
Neurologic	**Grade 1 Mild**	**Grade 2 Moderate**	**Grade 3 Severe**	**Grade 4**
Syncope		Loss of consciousness with no intervention required	Loss of consciousness AND intervention required	Hospitalization required

Respiratory	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4
Epistaxis	Intervention not indicated	Intervention required (e.g. nasal packing, topical vasoconstrictors)	Invasive intervention required (e.g., cauterization)	Transfusion

Table 10.3.1.1B. NCI-CTCAE Grading Criteria for Adverse Events Not Coded as an Allergic Reaction but not Described in Table 10.3.1.1A

Grade	Criteria
1	Mild
2	Moderate
3	Severe
4	Life-threatening or disabling adverse event
5	Death

10.3.1.2 Adverse Events Coded as Allergic Reactions

AEs coded as an allergic reaction will be graded according to the criteria set forth in the CoFAR Grading Scale defined in Table 10.3.1.2 below.

Table 10.3.1.2 CoFAR Grading Scale for Systemic Allergic Reactions Version 3.0

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Reaction involving **one of the following organ systems in which the symptoms are mild**: <u>Cutaneous</u> Generalized pruritus, generalized urticaria, flushing, angioedema <u>Upper respiratory</u> Rhinitis, cough unrelated to laryngeal edema or bronchospasm <u>Conjunctival</u> Injection/redness, itching, tearing <u>GI</u> Nausea, abdominal pain (no change in activity level), single episode of vomiting and/or single episode of diarrhea	Reaction involving **two or more of the following organ systems in which the symptoms are mild**: <u>Cutaneous</u> Generalized pruritus, generalized urticaria, flushing, angioedema <u>Upper respiratory</u> Rhinitis, cough unrelated to laryngeal edema or bronchospasm <u>Conjunctival</u> Injection/redness, itching, tearing <u>GI</u> Nausea, abdominal pain (no change in activity level), single episode of vomiting, and/or single episode of diarrhea **OR**	Reaction involving **one or more of the following organ systems**: <u>Lower respiratory</u> Throat tightness, wheezing, chest tightness, dyspnea, cough that respond to short-acting bronchodilator treatment (including IM epinephrine) with or without supplemental oxygen <u>GI</u> Severe abdominal pain, more than two episodes of vomiting and/or diarrhea	Life-threatening reaction involving **one or more of the following organ systems** with or without other symptoms listed in Grades 1 to 3: <u>Lower respiratory</u> Throat tightness with stridor, wheezing, chest tightness, dyspnea, or cough associated with a requirement for supplemental oxygen and refractoriness to short-acting bronchodilator treatment (including IM epinephrine)[1] **OR** Respiratory compromise requiring mechanical support	Death

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
	Reaction involving **at least one of the following organ systems in which the symptoms are moderate:** <u>Cutaneous</u> Generalized pruritus, generalized urticaria, flushing, angioedema <u>Upper respiratory</u> Rhinitis, cough unrelated to laryngeal edema or bronchospasm <u>Conjunctival</u> Injection/redness, itching, tearing <u>GI</u> Nausea, abdominal pain (with change in activity level), two episodes of vomiting and/or diarrhea		<u>Cardiovascular</u> Reduced blood pressure (BP) with associated symptoms of end-organ dysfunction (e.g., hypotonia [collapse], syncope) defined as: • Children: low systolic BP (age specific[2]) or >30% decrease in systolic BP • Adults: systolic BP of less than 90 mmHg or >30% decrease from baseline	

1. Examples of refractoriness could include continuous albuterol nebulizer or epinephrine IV infusion or more than three IM epinephrine injections.
2. Low systolic BP for children is defined as: less than 70 mmHg from 1 month to 1 year of age, less than (70 mmHg + [2 x age]) from 1 to 10 years of age, and less than 90 mmHg from 11 to 17 years of age.

10.3.2 Attribution Definitions

The relationship, or attribution, of an AE to a study procedure will be evaluated by the site investigator according to the criteria in Table 10.3.2 below. AEs that are clearly not related to a study procedure (Code 1) will not be entered into the study database. AEs that are possibly (Code 2) or definitely (Code 3) related to a study procedure will be entered into the study database on an AE case report form (CRF). If the investigator is unsure of the relatedness, the investigator shall consult with the DAIT Medical Monitor to determine attribution. Final determination of attribution for safety reporting will be made by DAIT Medical Monitor.

Table 10.3.2. Attribution of Adverse Events

Code	Descriptor	Relationship a Study Procedure
RELATED CATEGORIES		
2	Possible	The AE has a <u>reasonable possibility</u> to be related; there is evidence to suggest a causal relationship.
3	Definite	The AE is clearly related.

10.4 Collection and Recording of Adverse Events

10.4.1 Collection Period

AEs related to a study procedure will be collected from the time of the participant's first study procedure until the subject completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study.

10.4.2 Collecting Adverse Events

AEs (including SAEs) may be discovered through any of these methods:

- Observing the participant
- Interviewing the participant
- Receiving an unsolicited complaint from the participant
- In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an AE

10.4.3 Recording Adverse Events

AEs related to a study procedure will be entered into the study database by completion of an AE CRF.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the subject prematurely withdraws (without withdrawing consent) or is withdrawn from the study, whichever occurs first.

10.5 Reporting of Serious Adverse Events and Adverse Events

10.5.1 Reporting of Serious Adverse Events to DAIT/NIAID

This section describes the responsibilities of the site investigator to report SAEs to DAIT/NIAID via the reporting system established in this protocol. Timely reporting of AEs is required by ICH E6 guidelines.

Site investigators will report all SAEs within 24 hours of discovering the event.

For SAEs, all requested information on the AE/SAE CRF will be provided. However, unavailable details of the event will not delay submission of the known information. As additional details become available, the CRF will be updated and submitted.

10.5.2 Reporting of Adverse Events to Institutional Review Boards

AEs shall be reported in a timely fashion to the IRB(s) of record in accordance with applicable regulations and guidelines established by the IRB(s).

10.6 Reporting of Other Safety Information

An investigator shall promptly notify DAIT/NIAID and the SACCC within 24 hours of discovering an "unanticipated problem involving risks to subjects or others", which is not otherwise reportable as an AE.

10.7 Review of Safety Information

10.7.1 Medical Monitor Review

The DAIT/NIAID Medical Monitor shall receive reports from the SACCC, on a schedule to be determined by DAIT/NIAID, that compile new and accumulating information on AEs and SAEs recorded in the study database. In

addition, the DAIT/NIAID Medical Monitor shall review and make decisions on the disposition of the SAE reports received by the SACCC.

10.7.2 DSMB Review

10.7.2.1 Planned DSMB Reviews

The DSMB shall review safety data at least yearly during planned DSMB Data Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reportable AEs and SAEs. The DAIT/NIAID Medical Monitor will determine whether the reporting of an SAE to the DSMB should be expedited.

10.7.2.2 Ad hoc DSMB Reviews

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for *ad hoc* reviews. The DSMB will review any event that potentially impacts safety at the request of the protocol chair or DAIT. In addition, the following events will trigger an ad hoc comprehensive DSMB Safety Review:

- Any death that occurs in the study that is possibly or definitely related to a study procedure
- The occurrence of more than two AEs of Grade 4 or one Grade 5 event possibly or definitely related to an OFC
- The occurrence of more than one AE of Grade 4 or one of Grade 5 that is possibly or definitely related to a study procedure other than an OFC

After review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

10.7.2.2.1 Temporary Suspension for ad hoc DSMB Safety Review

Any study procedure that meets a study procedure stopping rule (Section 9.4 *Study Procedure Stopping Rules*) will be halted pending review by DAIT and the DSMB; however, all other study procedures may continue according to the Schedule of Events.

11 Statistical Considerations and Analytical Plan

11.1 Overview

The study objectives are restated here:

1. To study the role of established and novel clinical, environmental, biological, and genetic prenatal and early-life factors in the development of allergic diseases through age 3 years, with an emphasis on food allergy and atopic dermatitis.
2. To apply systems biology to identify mechanisms and biomarkers underlying the development of food allergy, atopic dermatitis, and their endotypes.
3. To collect, process, and assay or store environmental and biological samples for current and future use in the study of allergic disease development.

11.2 Primary Clinical Endpoints

The primary clinical endpoints are:

1. IgE-mediated, immediate-type allergy to protocol-specified foods assessed at each clinic visit, starting at the child's 5-month visit.
2. Atopic dermatitis assessed at each clinic visit starting at the child's 2-month visit.

11.3 Secondary Clinical Endpoints

The secondary clinical endpoints are:

1. Sensitization to protocol-specified foods assessed by serum IgE and skin prick testing at each clinic visit starting with the child's 5-month visit.
2. Sensitization to aeroallergens assessed by serum IgE and skin prick testing at the child's 12-, 24-, and 36-month clinic visits.
3. Recurrent wheeze assessed at the child's 36-month clinic visit.
4. Seasonal and perennial allergic rhinitis and allergic rhinoconjunctivitis assessed at the child's 36-month clinic visit.

11.4 Exploratory Endpoint

The exploratory endpoints are:

1. The resolution of allergy to individual foods, defined as the transition from allergic to non-allergic, assessed at the child's 12-, 24- and 36-month clinic visits.
2. IgE-mediated, immediate-type allergy to non-protocol-specified foods assessed at each clinic visit, starting at the child's 5-month visit.
3. Non-immediate-type food allergy (specifically, FPIAP and FPIES) and EoE assessed at each clinic visit, starting at the child's 5-month visit.

11.5 Analysis Plan

11.5.1 Analysis Populations

11.5.1.1 Full Cohort Population

The full cohort population will consist of all children ever enrolled.

11.5.1.2 Case-Cohort Populations

As participants are enrolled, 20% of the full cohort will be randomly assigned to a subcohort that will serve as a common control group for multiple case-control analyses (see Section 11.8.2 *Sample Size of the Subcohort*). For the analysis of a given clinical endpoint, the case-cohort population will consist of the subcohort (which will contain cases and non-cases) plus all cases that develop outside the subcohort. For diseases that produce a large number of cases, a sample of cases may be considered for the case-cohort sample. There will be a unique case-cohort population for each clinical endpoint analyzed with the case-cohort design; however, each case-cohort population will include the same subcohort.

11.5.2 Descriptive Analyses

For a given analysis using either the full cohort or the case-cohort population, characteristics of the biological mother, biological father, and/or child and distributions of exposure variables will be described with descriptive statistics, such as frequencies and percentages for categorical variables and means, medians, maximums and minimums, and interquartile ranges for continuous variables. Measures of incidence or prevalence will be reported for clinical endpoints.

11.5.3 Analysis of Associations Between Exposures and Endpoints

Associations between exposures (which includes risk factors and predictors) and primary, secondary, and exploratory clinical endpoints will be investigated in either the full cohort or the case-cohort population (See Section 11.5.4 *Analyses with the Case-Cohort Population*) with bivariate and multivariate statistical techniques. Analyses will include, but not be limited to, standard statistical techniques, such as the following:

- Chi-square and t-tests for categorical and continuous data, respectively
- Logistic regression for dichotomous outcomes, such as peanut allergy by age 12 month, yes or no
- Ordinal or multinomial logistic regression for categorical outcomes, such as atopic dermatitis by age 12 months categorized as severe, moderate, mild, or none
- Linear regression for continuous outcomes, such as SPT wheal sizes or IgE concentrations
- Poisson regression for endpoints that are counts, such as the number of food allergies or number of positive SPTs
- Cox regression for time-to-event analyses, such as the diagnosis of shellfish allergy by age 36 months
- Mixed linear models for multilevel, longitudinal, or correlated data

However, more advanced statistical techniques (e.g., random forests) may be conducted in addition to or in place of more standard techniques, especially for the analyses of mechanistic and genetic data.

The purpose of statistical modeling will typically be to investigate either etiology or prediction. The goal of etiologic modeling will be to identify potential causal factors and pathways and to measure the strength of unbiased associations, one of several criteria for evaluating causation. The goal of prediction modeling will be to create a model that maximizes discrimination between who will and will not develop the disease in question (or who does or does not have the disease in question).

11.5.4 Analyses with the Case-Cohort Population

Because a case-cohort analysis requires data only for the subcohort and the disease cases that arise outside of the subcohort, use of the design allows for a significant cost savings with regards to analyses that rely on data generated from biological samples. For the investigation of any given clinical endpoint, cases will be identified within the full cohort, which will include cases inside and outside of the subcohort, and non-cases will be identified inside of the subcohort. Various weighting methods have been proposed to make results for the case-cohort sample representative of the full cohort[52,57,58] (see a review and comparison of weighting methods in an article by Onland-Moret[59]). At the time of the analysis, the DAIT SACCC lead statistician and scientist will make recommendations to DAIT and the protocol chair on a weighting method. Sensitivity analyses may be conducted to evaluate the effects of the different weighting methods.

11.5.5 Missing Data Considerations

Missing data, which is a common problem in observational studies, especially observational studies that involve large numbers of subjects and assessments, can have a significant effect on conclusions drawn from an analysis. To minimize the amount of missing data, study staff and participants will be trained on the importance of data collection and prompts within the electronic data capture (EDC) system and electronic messaging will be used to remind study staff and participants to complete procedures. How missing data are handled in a given statistical analysis will depend on the study question under investigation, the statistical methods that will be conducted, the amount of missing data, and the nature of the missing data (e.g., missing completely at random, missing at random, or missing not at random). Generally, missing data will be handled by (1) deleting the missing observations from the analysis, (2) imputing the missing data, or (3) using statistical methods that can accommodate missing data, such as mixed linear

models. Sensitivity analyses may be conducted to investigate the effect of different methods of imputation. At the time of a given analysis, a plan for handling missing data will be developed by the SACCC lead statistician and scientist and presented to the protocol chair and DAIT/NIAID for approval.

11.6 Interim Analyses

The incidence of food allergy and atopic dermatitis will be calculated periodically during the recruitment period to assess the adequacy of the sample size. For sample size, a cumulative incidence for food allergy of 6% was assumed (Section 11.8 *Sample Size Considerations*), and it is expected that the cumulative incidence of atopic dermatitis will be higher than for food allergy (approximately 10%). Changes to the sample size may be proposed to the study's oversight boards for consideration.

Univariate statistics may be generated on any variable at any time during the study. Associations with disease endpoints may be analyzed at any timepoint after the full cohort has reached that timepoint; however, with approval by the SUNBEAM Steering Committee on a study-by-study basis, disease outcomes may be analyzed at a timepoint prior to the full cohort reaching that timepoint. For each study, statistical power and representativeness of a subset of the full cohort should be considered.

11.7 Mechanistic Analyses

Statistical analysis plans for the mechanistic investigations described in Section 7 *Planned Mechanistic Investigations and Assays* will be developed as part of detailed mechanistic protocols. It is anticipated that most mechanistic investigations will utilize a case-cohort population.

11.8 Sample Size Considerations

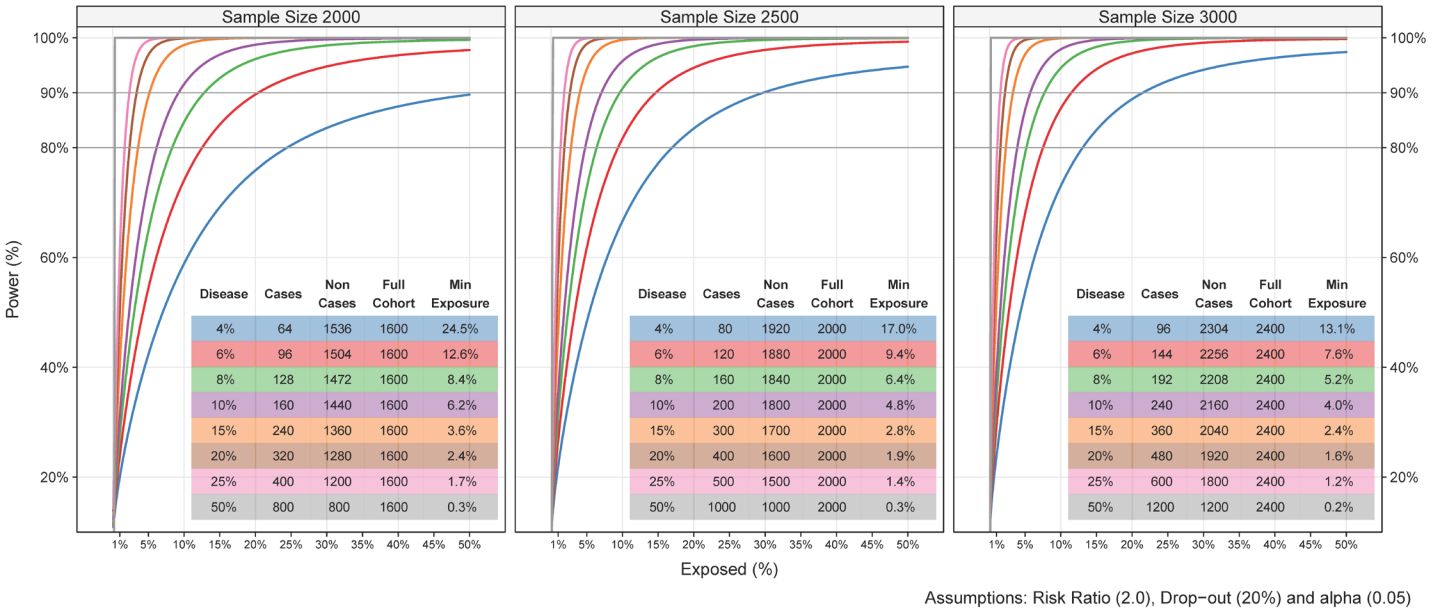
11.8.1 Sample Size of the Full Cohort

The sample size for a prospective cohort design is a function of the following:

- Statistical power
- The proportion of participants in the study sample with the exposure (or risk factor or predictor) of interest
  - Sample size is optimized at a risk factor proportion of 0.50 (i.e., 1:1)
  - Sample sizes increase sharply at risk factor proportions <0.30 and >0.70
- The overall risk of disease in the study sample
  - Less prevalent diseases require a larger sample size, e.g. investigations of food allergy questions will require a larger sample size than investigations of atopic dermatitis
- The size of the relative risk—smaller relative risks require larger sample sizes

The accrual objective is to enroll at least 2500 pregnant women and their offspring. Figure 11.8.1 shows power curves for 8 disease prevalences at 3 different cohort sizes. The tables embedded within the panels show the minimum exposure prevalences that can be investigated at 80% power. For a sample size of 2500, a prevalence of food allergy of 6% (the clinical endpoint with the lowest risk), a relative risk of 2.0, and a 20% drop-out rate, the study could investigate exposures with a prevalence as low as 9.4% at 80% power. It is important to note that for exposures that are rarer than 9.4%, the study will not have sufficient power to find statistically significant associations unless the relative risks are greater than 2.0 or the sample size is increased.

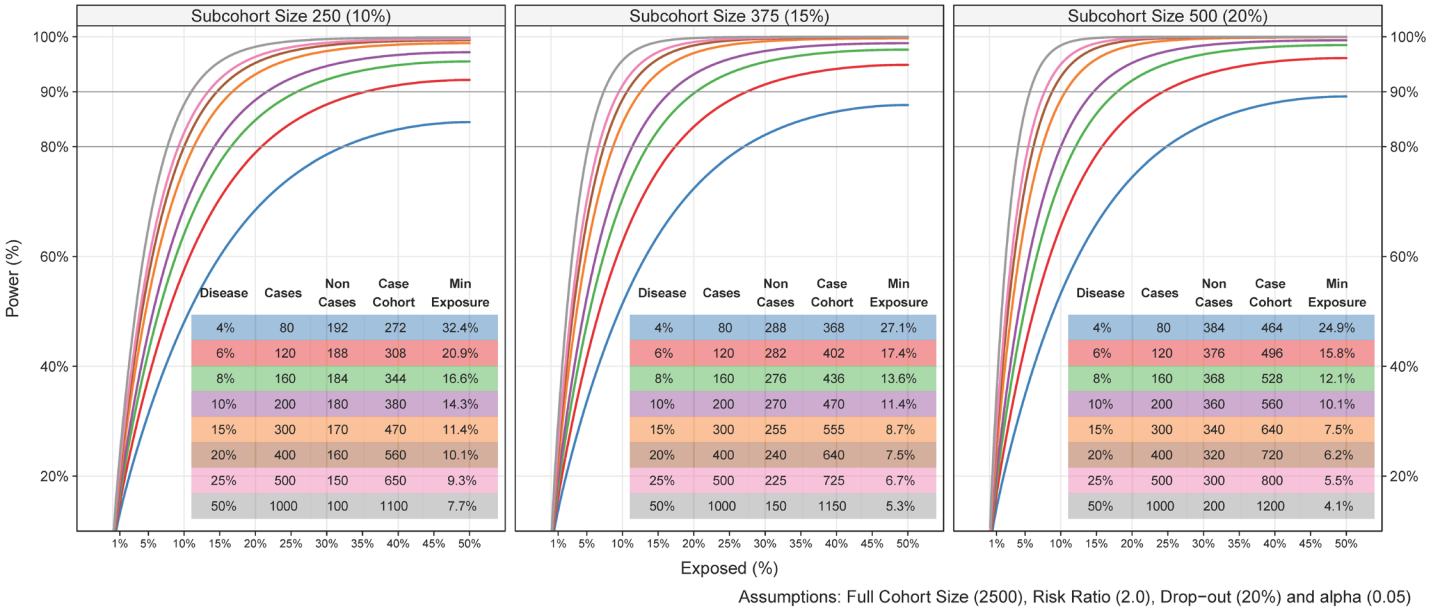
Figure 11.8.1. Full Cohort Sample Size



11.8.2 Sample Size of the Subcohort

Figure 11.8.2 shows power curves for 8 disease prevalences at 3 subcohort sizes. The tables embedded within the panels show the minimum exposure prevalences that can be investigated at 80% power. Sample size selection for the subcohort is a compromise between a smaller sample size that minimizes the number of biological samples that have to be assayed in a case-cohort analysis and a larger sample size that allows for the investigation of rare exposures among rare diseases. For this study, a subcohort sample size of 500 was selected because it allows for investigations of exposures with the smallest prevalences among the 3 sample size examples while staying within budgetary constraints for conducting sample assays. A subcohort size larger than 500 (not shown) does not provide sufficient gains in power to offset the additional assays that would be required. It should be noted that analyses using the full cohort of 2500 will have greater statistical power than analyses using the case-cohort design. For example, for the full cohort of 2500 and a disease prevalence of 6%, the minimum exposure prevalence that can be investigated at a relative risk of 2.0 and 80% power is 9.4% (Figure 11.8.1 *Full Cohort Sample Size*) compared to 15.8% for a case-cohort analysis based on a subcohort of 500.

Figure 11.8.2 Subcohort Sample Size



12 Identification and Access to Source Data

12.1 Source Data

Source documents and source data are considered to be the original documentation where subject information, visits consultations, examinations and other information are recorded. Documentation of source data is necessary for the reconstruction, evaluation and validation of clinical findings, observations and other activities during a clinical trial. In this study, source documentation may be paper or electronic, as when study staff enter participant's information directly into an electronic CRF or when a participant completes a web-based questionnaire.

12.2 Access to Source Data

The site investigators and site staff will make all source data available to DAIT/NIAID and SACCC personnel. Authorized representatives are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals.

13 Protocol Deviations

13.1 Protocol Deviation Definitions

Protocol Deviation – The investigators and site staff will conduct the study in accordance to the protocol; no deviations from the protocol are permitted. Any change, divergence, or departure from the study design or procedures constitutes a protocol deviation. As a result of any deviation, corrective actions will be developed by the site and implemented promptly.

Major Protocol Deviation (Protocol Violation) - A Protocol Violation is a deviation from the IRB approved protocol that may affect the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data. In addition, protocol violations include willful or knowing breaches of human subject protection regulations, or policies, any action that is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical

principles, and a serious or continuing noncompliance with federal, state, local or institutional human subject protection regulations, policies, or procedures.

Non-Major Protocol Deviation - A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that does not have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

13.2 Reporting and Managing Protocol Deviations

The study site PI has the responsibility to identify, document and report protocol deviations as directed by DAIT/NIAID. However, protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review.

Upon determination that a protocol deviation has occurred, the study staff will notify the site PI, the DAIT/NIAID Medical Monitor, and the SACCC of the deviation and will complete a Protocol Deviation form in the EDC system. If the classification of a deviation is in question, the site PI should consult with the DAIT/NIAID Medical Monitor. If there is not a consensus about the classification, the DAIT/NIAID Medical Monitor will make the final decision.

Protocol deviations will be reported to the IRB and DSMB at continuing reviews. The DAIT/NIAID Medical Monitor will determine whether the reporting of a major deviation to the IRB and DSMB should be expedited. For each major protocol deviation, a corrective plan will be developed by the site PI, the SACCC, and the DAIT/NIAID Project Manager and implemented site-wide or study-wide as required.

14 Ethical Considerations and Compliance with Good Clinical Practice

14.1 Quality Control and Quality Assurance

The site PI is required to keep accurate records to ensure that the conduct of the study is fully documented. The site PI is required to ensure that all CRFs are completed for every participant entered in the trial.

The Sponsor, DAIT/NIAID, is responsible for regular inspection of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data. On a frequency outlined in the Clinical Monitoring Plan, clinical monitors from the SACCC will conduct periodic reviews of the conduct of the study, either by in-person visits or remote data reviews.

The CRFs will be completed online via a web-based EDC system. Site staff will enter information into the electronic forms, and the data will be stored remotely at a central database. Data quality will be ensured through the EDC system’s continuous monitoring of data and real-time detection and correction of errors. All elements of data entry (e.g., time, date, verbatim text, and the name of the person performing the data entry) will be recorded in an electronic audit trail to allow all changes in the database to be monitored and maintained in accordance with federal regulations.

14.2 Statement of Compliance

This clinical study will be conducted in accordance with the *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*, and accordance to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by the IRB. Any amendments to the protocol or to the consent materials will also be approved by the IRB.

14.3 Informed Consent Process

For any informed consent described in this protocol, the consent may be obtained in person or remotely. Participants consented remotely will provide an electronic signature (see the MOP for the remote consenting). The consent process will provide information about the study to a prospective participant and will allow adequate time for review and discussion prior to his/her decision. The PI or designee will review the consent and answer questions. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. All participants (or their legally acceptable representative) will read, sign, and date a consent form before undergoing any study procedures. Consent materials will be presented in participants' primary language. A copy of the signed consent form will be given to the participant. If the father is participating only remotely, the father will be consented verbally and will not be required to sign a written consent form.

The consent process will be ongoing. The consent form will be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study.

14.4 Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a unique identification number and these numbers rather than names will be used to collect, store, and report participant information. Site personnel will not transmit documents containing personal health identifiers to DAIT, the SACCC, or their representatives.

15 Publication Policy

The CoFAR policy on the publication of study results will apply to this trial.

16 References

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17 APPENDIX A: Algorithm for Food Allergy Classification

17.1 General Instructions

The Food Allergy Algorithm consists of two diagrams—each on a separate page within Appendix A. Page 1 of the Food Allergy Algorithm uses food exposure, symptomology, SPTs, and OFCs to determine allergy status. Page 2 of the Food Allergy Algorithm incorporates food-specific IgE information to assist in very specific cases involving missing SPT or OFC data. When an OFC is indicted by the algorithm, investigators may use their clinical discretion not to perform the test. Wide clinic-visit windows (see Section 6.4.3 *Child's Clinic Visits and Visit Windows*) are provided to accommodate the need to reschedule any algorithm-based testing. The food allergy algorithm pertains to IgE-mediated allergy and is not applied for diagnosis of FPIAP, FPIES, or EoE.

The algorithm should be applied at ages 5, 12, 24, and 36 months to determine incident cases of allergy to protocol-specified foods and at ages 12, 24, and 36 months to determine the resolution of allergy to any foods. Baked forms of milk and egg will not be evaluated in the algorithm. Participants who are sensitized (i.e., positive skin test or serum IgE) and determined not to be allergic to a food will be encouraged to maintain that food in the diet.

Definitions of food exposure and symptoms used in the algorithm are provided below in Section 17.2 *Definitions*. For the determination of food allergy resolution, special rules for the application of the algorithm are provided below in Section 17.3 *Application of the Algorithm for Food Allergy Resolution*.

17.2 Definitions

Convincing history that exposure to food has been tolerated

This is operationally defined as the participant having ingested the food without indicating illness or reactions from the food EITHER at least 2 times at full serving sizes for age OR at least 1 time per week at amounts less than 1 serving size for age over the three months prior to the endpoint assessment.

Trace exposure

This is defined as a credible history of ingesting the food but in amounts or frequencies less than described above.

Convincing symptoms

This is defined as a credible history of an acute reaction within an hour of ingesting the food with at least one of the following signs or symptoms:

- Skin with hives and/or angioedema
- Respiratory symptoms: trouble breathing, wheezing, stridor, grunting, or throat tightness
- Repeated vomiting occurring within 1 hour of ingestion

17.3 Application of the Algorithm to Food Allergy Resolution

For children classified as allergic to a protocol-specified food at a prior visit, the Food Allergy Algorithm should be applied at the 12-month visit for milk, egg, and peanut and the 24- and 36-month visits for all protocol-specified foods to determine resolution of the food allergy. For any given child, the algorithm path will lead to a box that states 'Not Evaluable", "Allergic", "Not Allergic", or "OFC". To determine whether the child's classification for a given food should remain "Allergic" or be reclassified as "Resolved", the following rules should be applied:

If the Path Leads to "Not Evaluable"

- Retain classification of "Allergic" unless the prior diagnosis was made >12 months prior based only on SPT criteria (Algorithm Page 2). In the latter case, reclassify as "Not evaluable".

If the Path Leads to "Allergic"

- Retain classification of "Allergic" without conducting an OFC.

If the Path Leads to "Not Allergic"

- If convincing history since the last assessment that exposure has been tolerated, then reclassify as "Not Allergic", regardless of wheal size, without conducting an OFC.
- If a trace or no exposure since the last assessment and wheal ≤2 mm, then conduct an OFC.

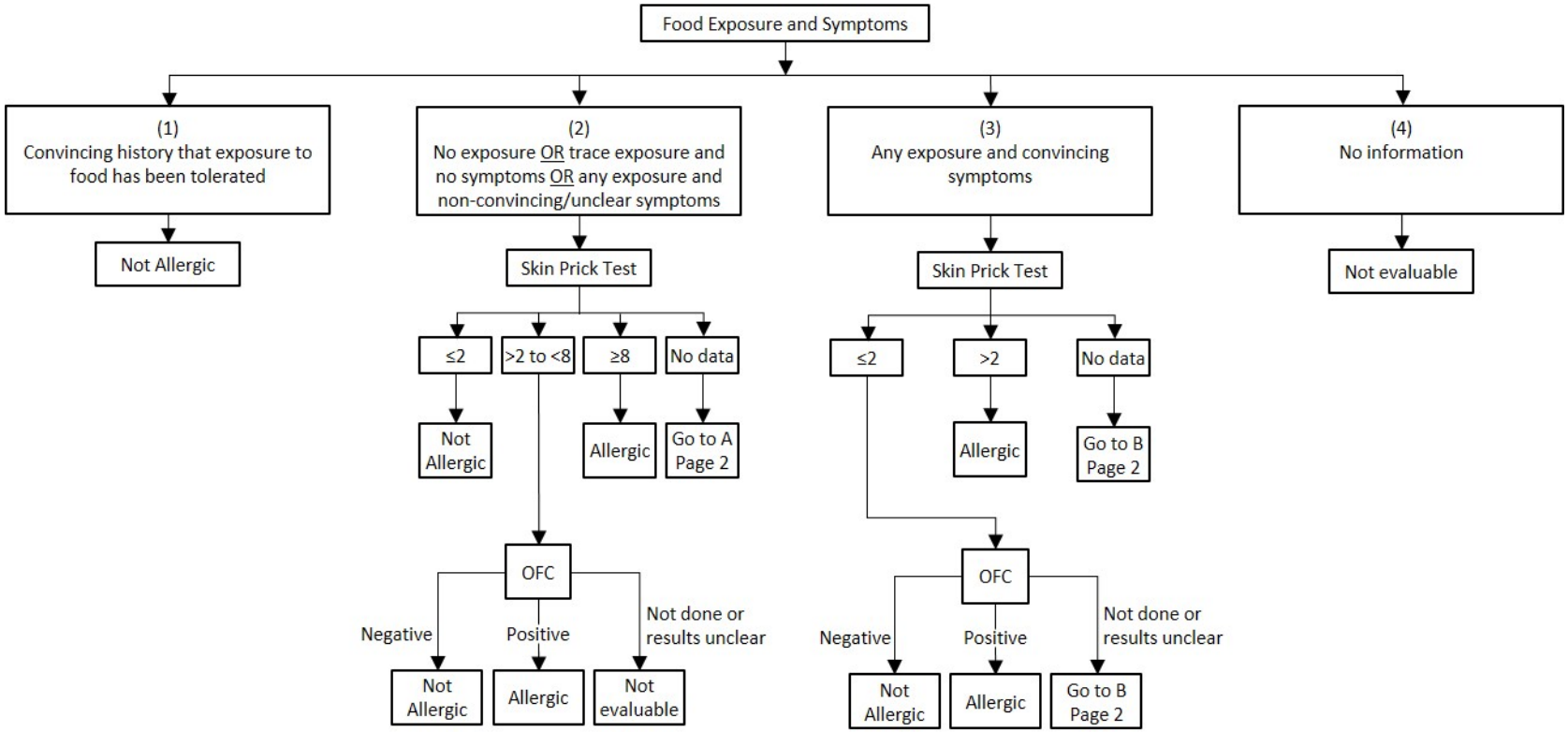
If the Path Leads to "OFC"

- For egg, milk, soy, and wheat:
  - 12-month visit (egg and milk only; ± visit window)
    - If no history of an allergic reaction ever to the specified food by either a positive OFC or parental report of convincing symptoms, then conduct an OFC.
    - If history of an allergic reaction to the specified food by either a positive OFC or parental report of convincing symptoms, then retain the classification of "Allergic".
  - 24-month visit (egg, milk, soy, and wheat; ± visit window):
    - If no history of an allergic reaction ever to the specified food by either a positive OFC or parental report of convincing symptoms, then conduct an OFC.
    - If history of an allergic reaction to the specified food by either a positive OFC or parental report of convincing symptoms at or prior to the 12-month visit (± visit window), then conduct an OFC.
    - If history of an allergic reaction to the specified food by either a positive OFC or parental report of convincing symptoms more recent than the 12-month visit (± visit window), then retain the classification of "Allergic".
  - 36-month visit (egg, milk, soy, and wheat; ± visit window):
    - If no history of an allergic reaction ever to the specified food by either a positive OFC or parental report of convincing symptoms, then conduct an OFC.
    - If history of an allergic reaction to the specified food by either a positive OFC or parental report of convincing symptoms at or prior to the 24-month visit (± visit window), then conduct an OFC.
    - If history of an allergic reaction to the specified food by either a positive OFC or parental report of convincing symptoms more recent than the 24-month visit (± visit window), then retain the classification of "Allergic".

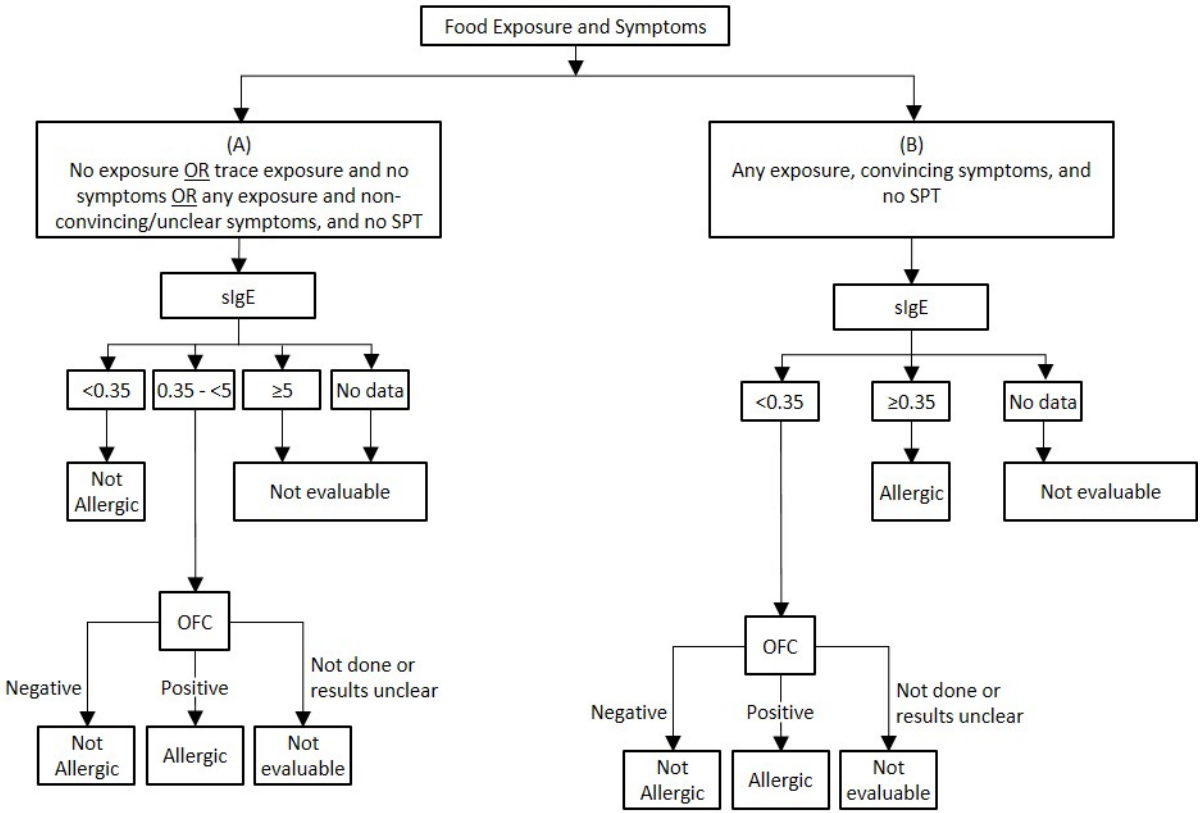
- For peanut, fish, shell fish, tree nut, and sesame:
  - 12-month visit (± visit window):
    - If no history of an allergic reaction ever to the specified food by either a positive OFC or parental report of convincing symptoms, then conduct an OFC.
    - If history of an allergic reaction to the specified food by either a positive OFC or parental report of convincing symptoms, then retain the classification of "Allergic".

- 24-month visit (± visit window):
  - If no history of an allergic reaction ever to the specified food by either a positive OFC or parental report of convincing symptoms, then conduct an OFC.
  - If history of an allergic reaction to the specified food by either a positive OFC or parental report of convincing symptoms at any time prior to the 24-month visit (± visit window), then retain the classification of “Allergic”.
- 36-month visit (± visit window):
  - If no history of an allergic reaction ever to the specified food by either a positive OFC or parental report of convincing symptoms, then conduct an OFC.
  - If history of an allergic reaction to the specified food by either a positive OFC or parental report of convincing symptoms at or prior to the 12-month visit (± visit window), then conduct an OFC.
  - If history of an allergic reaction to the specified food by either a positive OFC or parental report of convincing symptoms more recent than the 12-month visit (± visit window), then retain the classification of “Allergic”.

Food Allergy Algorithm Page 1



Food Allergy Algorithm Page 2



18 APPENDIX B: Diagnosis of Food Protein-Induced Enterocolitis Syndrome (FPIES)

History of adverse reactions to foods may disclose symptoms consistent with acute or chronic FPIES. The diagnostic criteria[55] here suggests food challenges be strongly considered in cases with a single episode. These food challenges will not be undertaken as part of this study and are not required to record an FPIES diagnosis if there is one episode with 3 minor criteria met and no suspicion of a viral infection by history.

<table>
<tr><td colspan="2">Acute FPIES</td></tr>
<tr><td>Major criterion:

Vomiting in the 1- to 4-hour period after ingestion of the suspect food and absence of classic IgE-mediated allergic skin or respiratory symptoms</td><td>Minor criteria:

1. A second (or more) episode of repetitive vomiting after eating the same suspect food

2. Repetitive vomiting episode 1-4 h after eating a different food

3. Extreme lethargy with any suspected reaction

4. Marked pallor with any suspected reaction

5. Need for emergency department visit with any suspected reaction

6. Need for intravenous fluid support with any suspected reaction

7. Diarrhea in 24 h (usually 5-10 h)

8. Hypotension

9. Hypothermia</td></tr>
<tr><td colspan="2">The diagnosis of FPIES requires that a patient meets the major criterion and ≥3 minor criteria. If only a single episode has occurred, a diagnostic OFC should be strongly considered to confirm the diagnosis, especially because viral gastroenteritis is so common in this age group. Furthermore, although not a criterion for diagnosis, it is important to recognize that acute FPIES reactions will typically completely resolve over a matter of hours compared with the usual several-day time course of gastroenteritis. The patient should be asymptomatic and growing normally when the offending food is eliminated from the diet.</td></tr>
<tr><td colspan="2">Chronic FPIES</td></tr>
<tr><td>Severe presentation: When the offending food is ingested on a regular basis (e.g., infant formula); intermittent but progressive vomiting and diarrhea (occasionally with blood) develop, sometimes with dehydration and metabolic acidosis.
Milder presentation: Lower doses of the problem food (e.g., solid foods or food allergens in breast milk) lead to intermittent</td><td>The most important criterion for chronic FPIES diagnosis is resolution of the symptoms within days after elimination of the offending food(s) and acute recurrence of symptoms when the food is reintroduced, onset of vomiting in 1-4 h, diarrhea in 24 h (usually 5-10 h). Without confirmatory challenge, the diagnosis of chronic FPIES remains presumptive.</td></tr>
</table>

Chronic FPIES	
vomiting and/or diarrhea, usually with poor weight gain/FTT but without dehydration or metabolic acidosis.	