

URECA

Urban Environment and Childhood Asthma

NIAID Protocol Number: ICAC-07

**Sponsored by:
National Institute of Allergy and Infectious Diseases (NIAID)**

Principal Investigator: James E. Gern

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Protocol Addendum
Continuation of URECA Study to Age 14/16**

17 October 2019

Statement of Compliance

This clinical study will be conducted using good clinical practice (GCP), as delineated in Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance, and according 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 central IRB. Any amendments to the protocol or to the consent materials will also be approved by the central IRB before they are implemented.

TABLE OF CONTENTS

Statement of Compliance.....	2
Protocol Summary	7
Schematic of Study Design.....	9
Key Roles	9
1 Background Information and Scientific Rationale	11
1.1 Background Information.....	11
1.2 Rationale.....	14
1.3 Potential Risks and Benefits	14
1.3.1 Risks of Study Procedures	14
1.3.1.1 Allergy Skin Testing.....	15
1.3.1.2 Blood Collection.....	15
1.3.1.3 Spirometry	15
1.3.1.4 Methacholine Challenge	15
1.3.1.5 Sputum Induction.....	16
1.3.1.6 Exhaled Nitric Oxide Measurement	16
1.3.1.7 Nasal Epithelial Cell Collection	16
1.3.1.8 Questionnaires.....	16
1.3.2 Potential Benefits	16
2 Study Objectives.....	16
2.1 Primary Objective	16
2.2 Secondary Objectives.....	16
2.3 Exploratory Objectives	17
3 Study Design	17
3.1 Description of the Study Design.....	17
3.2 Study Endpoints.....	18
3.2.1 Primary Endpoint.....	18
3.2.2 Secondary Endpoints	18
4 Study Population.....	18
4.1 Population Description.....	19
4.2 Participant Inclusion Criteria	19
5 Study Procedures/Evaluations.....	19
5.1 Clinical Evaluations.....	20
5.1.1 Dust Analysis for Allergen and Microbial Products.....	20
5.1.2 Allergen Skin Testing	20
5.1.3 Pre/Post Bronchodilator Pulmonary Function Testing	20
5.1.4 Methacholine Challenge.....	20
5.1.5 Exhaled Nitric Oxide Measurement.....	21
5.1.6 Induced Sputum	21
5.1.7 Nasal Epithelial Cell Collection.....	21
5.1.8 Fitness Testing.....	21
5.2 Laboratory Evaluations	21
5.2.1 Clinical and Research Laboratory Evaluations and Specimen Collection.....	21
5.2.1.1 Urine Pregnancy Testing	21
5.2.1.2 Urine Collection	22
5.2.1.3 Blood Collection.....	22

TABLE OF CONTENTS

5.2.1.4	Nasal Fluid.....	22
5.2.1.5	Immunologic Studies	22
5.2.2	Specimen Preparation, Handling and Shipping.....	22
5.2.2.1	Instructions for Specimen Storage.....	22
5.2.2.2	Specimen Shipment.....	23
5.3	Substudies	23
6	Research Use of Stored Human Samples, Specimens or Data.....	23
6.1	Use of Stored Samples/Data	23
6.2	Disposition of Stored Samples/Data	24
7	Study Schedule.....	24
7.1	Informed Consent	24
7.2	11-Year Clinic Visit	24
7.3	12-Year Clinic Visit	24
7.4	13-Year Clinic Visit	24
7.5	14-Year Clinic Visits.....	25
7.6	15-Year Clinic Visit	25
7.7	16-Year Clinic Visit	25
7.8	Induced Sputum Visit.....	25
7.9	Quarterly Phone Calls.....	25
7.10	Home Environmental Exposure Evaluation	26
7.11	Additional Visits or Contacts	26
7.12	Recontact of Subjects after Study Termination	26
8	Assessment of Safety	26
8.1	Definition of an Adverse Event (AE)	27
8.2	Definition of a Serious Adverse Event (SAE).....	27
8.3	Death Not Related to Study Procedures.....	27
8.4	Methods and Timing for Assessing, Recording, Analyzing and Adverse Events	28
8.4.1	Methods and Timing for Assessment	28
8.4.1.1	Grading of Adverse Events/Serious Adverse Events.....	28
8.4.1.2	Attribution of Adverse Event to Study Procedure.....	28
8.4.2	Recording/Documentation	29
8.4.3	Analysis/Management.....	29
8.5	Reporting Procedures.....	29
8.5.1	Serious Adverse Event Reporting	29
8.5.2	Adverse Event Reporting	29
8.5.3	Reporting to the IRB.....	30
8.5.4	Reporting Pregnancy.....	30
8.6	Type and Duration of the Follow-up of Participants after Adverse Events 30	
8.7	Participant Discontinuation	30
8.8	Replacement of a Participant Who Discontinues Study Participation	30
9	Clinical Monitoring Structure.....	31
9.1	Site Monitoring Plan.....	31
9.2	Safety Monitoring.....	31
9.2.1	Medical Monitor Review	31

TABLE OF CONTENTS

9.2.2	DSMB Review	31
10	Statistical Considerations	31
10.1	Overview	31
10.2	Endpoints	32
10.2.1	Primary Endpoints.....	32
10.2.2	Secondary Endpoints.....	32
10.3	Measures to Minimize Bias.....	32
10.4	Analysis Plan	33
10.4.1	Analysis Population.....	33
10.4.2	Primary Analysis of Primary Objective	33
10.4.3	Analysis of Secondary Objectives	34
10.4.3.1	Lung function	34
10.4.3.2	Urban exposures related to asthma, allergic sensitivity and allergic rhinitis	34
10.4.3.3	Effect of pubertal changes in adiposity and sex hormones on lung growth and asthma onset, prevalence and morbidity	35
10.4.4	Analysis of Exploratory Endpoints.....	36
10.5	Sample Size Considerations.....	36
11	Quality Control and Quality Assurance	36
12	Ethics/Protection of Human Subjects	36
12.1	The Belmont Report	36
12.2	Institutional Review Board	37
12.3	Informed Consent Process	37
12.4	Assent Process.....	37
12.5	Participant Confidentiality	37
12.6	Study Discontinuation.....	38
13	Data Handling and Record Keeping.....	38
13.1	Data Management Responsibilities	38
13.2	Data Capture Methods	39
13.3	Types of Data	39
13.4	Source Documents and Access to Source Data/Documents.....	39
13.5	Timing/Reports	39
13.6	Study Records Retention.....	39
14	Publication Policy	39
	Appendix A: Scientific References	40
	Appendix B: Schedule of Procedures/Evaluations	42

List of Abbreviations

ACRN	Asthma Clinical Research Network
AE	Adverse Event/Adverse Experience
ANOVA	Analysis of Variance
APIC	Asthma Phenotypes in the Inner City (trial)
ATS	American Thoracic Society
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
CFR	Code of Federal Regulations
CRF	Case Report Form
CMP	Clinical Monitoring Plan
DAIT	Division of Allergy, Immunology, and Transplantation
DMP	Data Management Plan
DNA	Deoxyribonucleic Acid
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
eNO	Exhaled Nitric Oxide
FDA	Food and Drug Administration
FEV ₁	Forced Expiratory Volume in one second
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
ICAC	Inner City Asthma Consortium
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IOS	Impulse Oscillometry
IRB	Institutional Review Board
LCA	Latent Class Analyses
MDI	Metered-Dose Inhaler
MOP	Manual of Operations
mRNA	Messenger RNA (ribonucleic acid)
N	Number (typically refers to participants)
NHLBI	National Heart, Lung, and Blood Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
PBMC	Peripheral Blood Mononuclear Cells
PD ₂₀	Provocative Dose that causes a 20% fall in FEV ₁
PI	[Site] Principal Investigator
RNA	Ribonucleic Acid
SACCC	Statistical and Clinical Coordinating Center
SAE	Serious Adverse Event/Serious Adverse Experience

Protocol Summary

Full Title:	Urban Environment and Childhood Asthma
Short Title:	URECA
Conducted by:	National Institute of Allergy and Infectious Diseases
Principal Investigator:	James Gern, MD
Sample Size:	N=609 originally enrolled
Study Population:	Inner-city children at high risk for development of asthma due to family history, plus a small sample of inner-city children without family history.
Accrual Period:	NA – Study participants were accrued from 2005-2007
Study Design:	URECA is a longitudinal birth cohort study which is being extended to follow study children to age 14/16
Study Duration:	Start Date: 1 May 2015 End Date: 31 July 2021
Primary Objective:	To determine the wheezing, asthma and atopy phenotypes in minority children growing up in poor urban neighborhoods as they develop from birth through adolescence.
Secondary Objectives:	<ol style="list-style-type: none">1. To longitudinally analyze the development of lung function and its relationship to asthma.2. To identify which urban exposures (allergens, pollutants, microbes, viral infections, stress) from early life onward affect the development and natural history through adolescence of asthma, allergic rhinitis and allergic sensitization.3. To determine the association of pre-pubertal obesity and the effect of pubertal changes in adiposity and sex hormones on lung growth and asthma onset, prevalence and morbidity.
Exploratory Objectives:	<ol style="list-style-type: none">1. To provide samples for analysis and outcomes data for use in mechanistic studies to understand relationships between environmental exposures, changes in immune development, and the

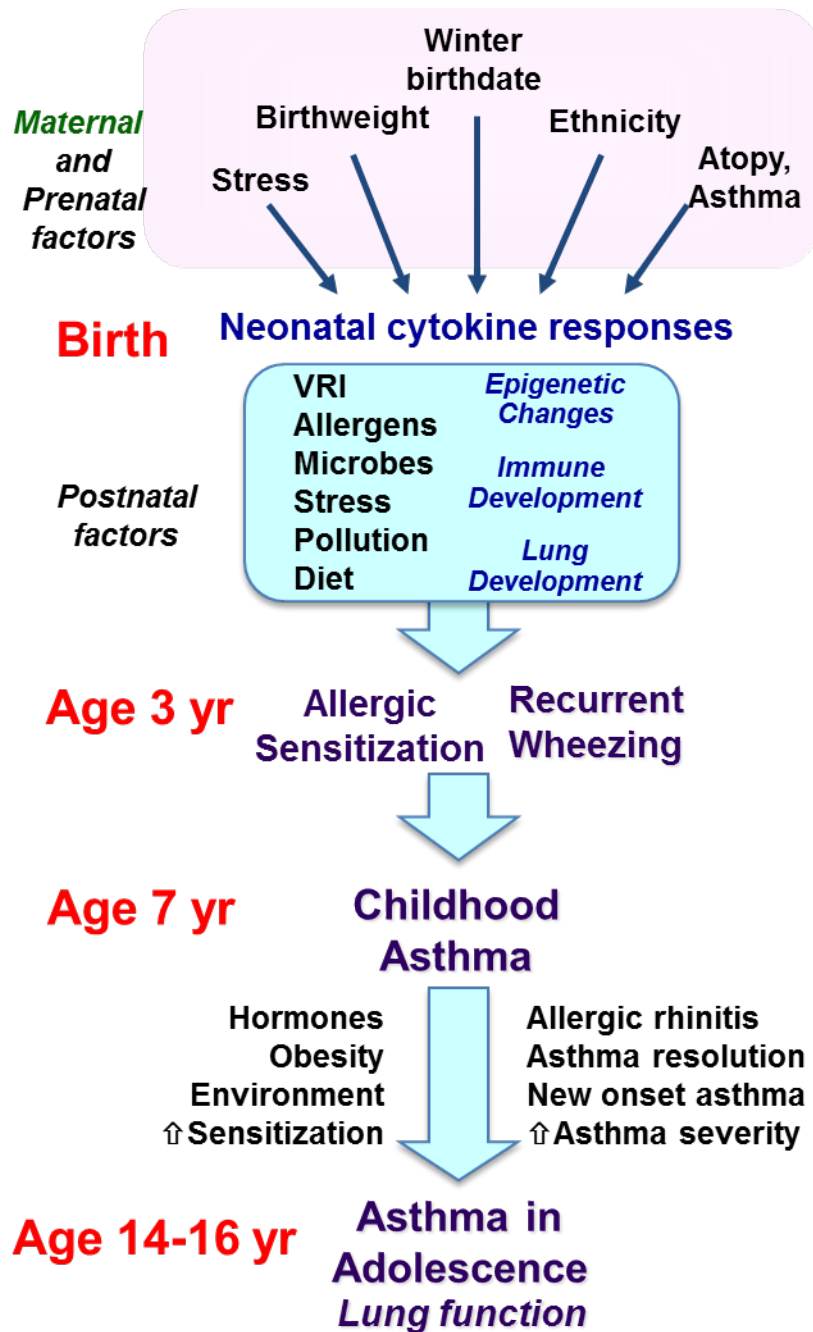
development of allergic sensitization, allergic rhinitis and asthma.

2. To work collaboratively with investigators outside of the ICAC network on mechanistic or clinical studies to test novel hypotheses and to validate, compare and contrast findings from URECA with those in other cohorts or clinical studies.

Endpoints:

Incidence of asthma and occurrence of specific phenotypes of asthma

Schematic of Study Design



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1 Background Information and Scientific Rationale

1.1 Background Information

The Urban Environment and Childhood Asthma (URECA) birth cohort study was initiated in 2004 by the Inner City Asthma Consortium (ICAC) to conduct comprehensive studies to define early life exposures that promote the development of disturbances in immune function that will lead to allergic sensitization and asthma.^{1,2} The study recruited a cohort of children from urban areas in economically disadvantaged neighborhoods in New York City, Boston, Baltimore, and St. Louis. The overall goal of these studies has been to gain greater insight into the unique effects of the inner city environment on the development of asthma, and from these findings identify more effective treatments in this high risk group of children and to begin efforts to prevent the expression of asthma in inner city youngsters. The study design focused on collecting data on key environmental exposures (stress, viral infections, allergens, microbes, indoor pollutants), measures of immune development (PBMC cytokine responses, T regulatory cells, allergen-specific IgE and IgG, plasma cytokines), and indicators of clinical outcomes (wheezing episodes, allergic sensitization, medication use, lung function testing). To accomplish these goals, study participants had yearly clinic visits with quarterly phone calls in between. Home visits were scheduled at intervals for environmental sampling including dust collection; after age 5 years, parents participated in the environmental sampling by collecting vacuumed dust.

The URECA study to date has had great success in a number of areas that are critical to establishing the cohort and understanding the pathogenesis of childhood asthma. The clinical teams collaborated with community leaders and obstetrical groups to recruit a study group representing mothers and children (70% Black, 20% Latino or Hispanic) residing in urban areas of poverty and difficult living circumstances that are reflective of US inner cities.² Both recruitment and retention were highly successful. In fact, successful recruitment strategies enabled us to **exceed the initial targeted sample size**. Despite a historic lack of connection to the medical establishment and atmosphere of distrust of participation in clinical research, the clinical teams used frequent contacts, neighborhood resources, home visits, and an attitude of inclusiveness to maintain retention rates that has far exceeded expectations;³ it is notable that **78% of the children (now ages 8-9 years) remain active participants** (Figure 1, data from October 2014). Through our recruitment efforts and frequent communication between study staff and participants,

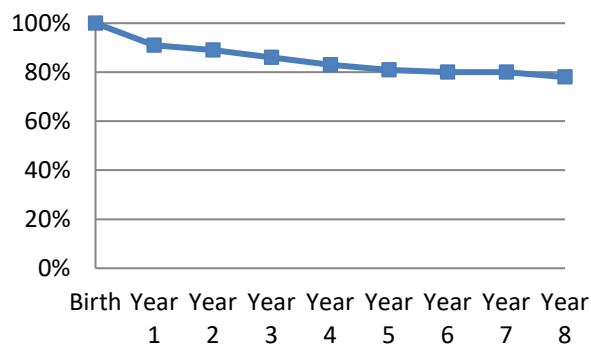


Figure 1. URECA retention through 10/06/14

we have established a community awareness that will have subsequent benefits in any efforts to intervene and reduce incidence and morbidity of asthma.

The hypothesis for the initial phase of the study (prenatal to age 3 years) was that **early exposure to adverse environmental factors such as cockroach allergens and stress would adversely affect immune development (as measured by mononuclear cell cytokine responses), and thereby promote the development of atopy and recurrent wheezing by age 3 years** (Figure 1). To date, we have identified significant relationships between a) prenatal perinatal exposures (stress, season of birth) and immune development, b) immune responses at birth and risk of developing atopic conditions in early life, and most recently, c) relationships between early life exposures (allergens, microbes, air quality, stress) and the risk of recurrent wheeze, atopy and lung function by age 3 years.⁴ Remarkably, in this initial phase of study, although we found that cumulative allergen exposure over the first 3 years of life was associated with allergic sensitization and that allergic sensitization was associated with recurrent wheeze, levels of cockroach, mouse and, to a lesser extent, cat allergen in the first-year inner city house dust samples were *inversely* related with recurrent wheeze at age 3 years. Effects of exposure to these allergens were additive (Figure 2A), and an “exposure index” to these three allergens was inversely related to wheeze over the entire range of exposure (Figure 2B), and this effect was independent of the atopic status of the child (Figure 2C). This inverse relationship was strongest for allergen levels in the first year dust samples, suggesting that the first few months of life is a critical time period in childhood allergic disease development.

First-year house dust samples from a nested case-control sample of 104 URECA children who were stratified into four groups (atopy alone, recurrent wheeze alone, both, or neither) based on their status at age 3 years were further analyzed for bacterial taxa using an array-based technique (PhyloChip-3). Combined analysis of first year exposure to both allergens and bacteria revealed that the group of children with neither wheeze nor atopy had the *highest* exposure to both selected allergens (cockroach, mouse and cat) and bacterial richness (Figure 3). These novel findings suggest that exposure to selected allergens and microbes in early life may *reduce* the risk of allergy and wheezing illnesses, even in the context of the high-risk urban environment.

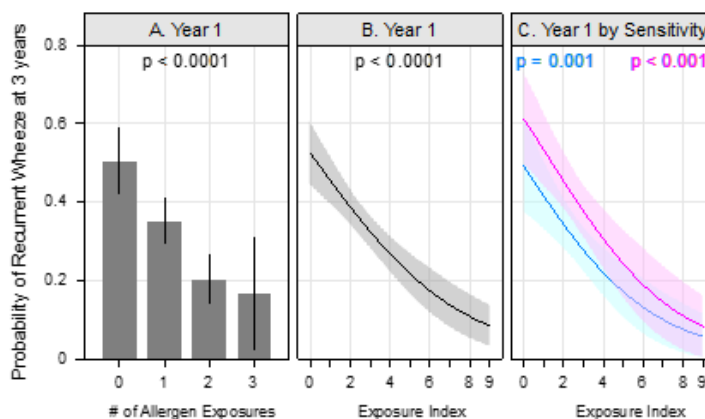


Figure 2. Association between allergen exposure and prevalence of recurrent wheeze at age 3 (see text).

In addition to findings related to exposures to allergens and microbes, several other important findings have emerged from the analysis of the data set through age 3-5 years. These findings include the following:

- At age 3 years, 44% of the main high-risk cohort were sensitized to at least one aeroallergen and 36% met the criteria for recurrent wheeze,⁴
- Maternal stress (OR 1.10; 95% CI: 1.03 – 1.17) and depression (OR 1.07; 95% CI: 1.04 – 1.11) are positively related to recurrent wheeze at age 3,
- Distinct patterns of innate and adaptive immune responses of PBMC are associated with a) environmental exposures (allergens, endotoxin and ergosterol), and b) the onset of atopy and recurrent wheeze,
- URECA children have a high rate (29% by age 3 years) of overweight status (BMI ≥ 85%) which may lead to predisposition to wheezing illnesses,
- Through age 5 years, 55% of the cohort were sensitized to one or more of the 3 target food allergens (milk 46%, egg 31%, peanut 21%), while 9.9% were diagnosed with food allergy (peanut 6.0%, egg 4.3%, milk 2.7%, 2.5% >1 food). Food sensitization was inversely related to mouse allergen exposure in the first year,⁵
- Vitamin D levels in cord blood were generally low (mean 20.5 ± 9.7 ng/mL) in this population, but did not predict an increased risk of wheezing in infancy, and
- Comparison of lung function measurements obtained at 3 and 4 years of age demonstrate high rates of acceptable maneuvers for spirometry (77% and 86%) and impulse oscillometry (IOS; 58% and 85%). Furthermore, wheeze was significantly associated with changes in IOS and spirometry measurements by age 4 years.

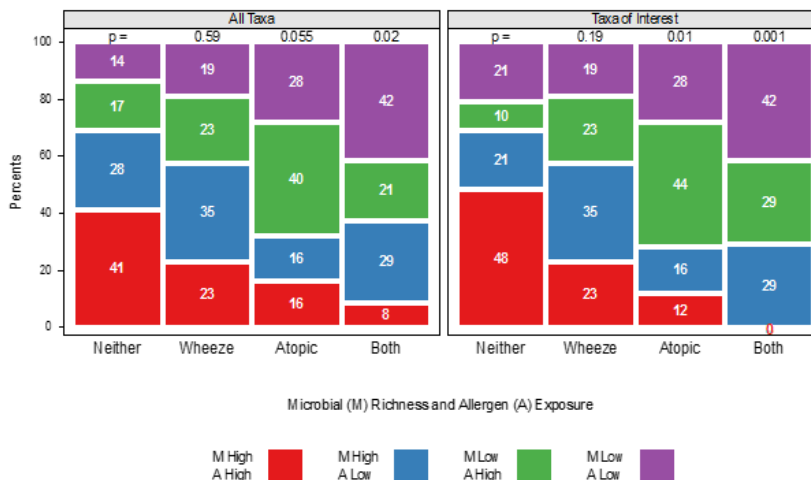


Figure 3. Combined effects of first-year exposure to allergens and microbes on atopy and wheeze.

The study has amassed a wealth of information that will be used to define urban risk factors for atopy, wheezing illnesses, and ultimately, allergic rhinitis and asthma. Previous studies of asthma in the inner-city environment have been cross-sectional in design. In URECA, prospective collection of samples began at birth, before signs of disease were manifest, and longitudinal assessments were performed to enable analysis of long-term and cumulative exposures beginning at birth. This unique comprehensive and longitudinal data set of environmental exposures include measurements of exposure to allergens and

microbes, family and neighborhood stress (beginning in the prenatal period), viral infections and illness, indoor pollutants, and consumption of key nutrients.² Serial immunologic measurements of innate and adaptive immune responses, designed with assistance from the Immune Tolerance Network, were initiated at birth and have been assessed every 1-2 years.⁶ Lung function measurements were initiated at a very young age (33-36 months) with high rates of success and quality, and are repeated yearly. **Collectively these measurements constitute a unique and longitudinal data set to define urban environmental determinants of immune development, and effects on the development of atopy, wheeze, lung function and asthma during the critical formative period in early childhood.**

The URECA II protocol was completed in June 2014, with the last of the 7-year clinic visits. Analyses to determine environmental and immunologic predictors of the primary asthma outcome are ongoing, but preliminary analysis show that 29% of the high-risk cohort met the criteria for asthma and that 62% were sensitized to at least one aeroallergen. The novel findings to age 5 years, excellent retention, and high prevalence of the outcomes at age 7 years attest to the validity of the study design, which presents an outstanding opportunity to pose additional questions related to the origins and evolution of asthma in inner city children.

1.2 Rationale

Ongoing activities in the URECA protocol are designed to identify environmental risk factors that modify immune development and the onset of allergic diseases and asthma. As the children participating in the study mature, activities in URECA will be expanded to address three main themes related to allergic diseases and asthma: onset and prevention, progression, and treatment and response. The next phase of URECA will follow children from age 10 years to ages 14-16 years, and offers additional opportunities for precise characterization, classification, phenotyping, and possibly endotyping of natural or longitudinal history of asthma among the study participants. We will focus on the mechanisms driving the changes in asthma prevalence and severity during adolescence, as well as the onset and evolution of allergic rhinitis during this same time period.

The results of the URECA birth cohort study and associated mechanistic protocols are likely to provide the basis for new preventive and therapeutic strategies for health disparities related to childhood asthma in poor urban areas.

1.3 Potential Risks and Benefits

1.3.1 Risks of Study Procedures

The URECA study has been designated as an observational birth cohort study by the ICAC and DAIT. As such, this study involves minimal active intervention outside of the normal standard of care for the participant. No study drugs are used in the URECA study. All risks of the URECA study are limited to study procedures, i.e. blood draws, nasal

sample collection, allergen skin testing, sputum collection, or pulmonary function tests not included as part of standard of care for this group of patients.

1.3.1.1 Allergy Skin Testing

If the participant is allergic to any of the allergens, redness, swelling, and itching of the skin may occur and last for 1 to 2 hours. These symptoms could occur up to one or two days after the skin test. The study doctor may provide oral or topical antihistamines to treat these symptoms. There is also a very rare chance that the participant may have asthma symptoms or faint during the test. A medical provider trained in treating anaphylaxis will be available to provide immediate treatment in the event that a participant experiences an allergic reaction. Stopping antihistamines before skin testing may make allergy (but not asthma) symptoms worse. Participants will be told they can take their medications if they need them, but the test will need to be rescheduled.

1.3.1.2 Blood Collection

The risks associated with taking blood include possible pain from the stick, as well as bleeding, bruising, and infection of the skin. Lightheadedness and fainting rarely occur during non-fasting blood collections, but are more likely during fasting blood collections. To minimize these risks, a staff member who is trained to draw blood from children will collect the samples. Additionally, investigative sites may apply an analgesic medication such as EMLA® to the skin before the blood draw to reduce the pain of the stick. Side effects from this medication include erythema, burning, paleness at the skin site, edema, and alterations in temperature. Reactions are mild and transient. There is a potential for allergic reactions.

1.3.1.3 Spirometry

Spirometry can cause coughing or lightheadedness, which will go away shortly after the test is finished. The albuterol that is given during reversibility testing can cause increased heart rate and blood pressure, nausea, headache, and a jittery or nervous feeling. These symptoms usually resolve in less than an hour.

Participants will be asked to withhold their asthma medications for a period of time before the procedure depending on the medication. Withholding of asthma medications before testing may cause a worsening of asthma symptoms. Participants will be informed that they can take their asthma medications if needed, and the procedure will be rescheduled. Exact medications to withhold, length of abstention and procedures are described in the URECA IV Manual of Operations (MOP) for ICAC-07.

1.3.1.4 Methacholine Challenge

Methacholine challenge can cause coughing, chest tightness, shortness of breath, and wheezing. A medication to open the airways (albuterol) will be promptly given to help reverse these effects. There is a rare risk that a severe asthma episode may occur. A study clinician experienced in asthma care will be available should such an episode occur.

Participants will be asked to withhold their asthma medications for a period of time before the procedure, depending on the medication. Withholding of asthma medications before testing may cause a worsening of asthma symptoms. Participants will be informed that they can take their asthma medications if needed, and the procedure will be rescheduled. Exact medications to withhold, length of abstention and procedures are described in the URECA IV MOP for ICAC-07.

1.3.1.5 Sputum Induction

Sputum induction may result in wheezing, coughing or chest tightness. Participants will be pre-medicated with albuterol in order to minimize this risk.

1.3.1.6 Exhaled Nitric Oxide Measurement

This test may cause dizziness which will go away soon after the test is finished.

1.3.1.7 Nasal Epithelial Cell Collection

The risks associated with the nasal cell collection procedure include discomfort or pain, transient nosebleed, sneezing, tearing of the eyes, runny nose, and postnasal drip.

1.3.1.8 Questionnaires

There is a possibility that participants may find the questions too personal. Participants may refuse to answer any questions that make them feel uncomfortable.

1.3.2 Potential Benefits

Those participants with asthma may benefit by receiving frequent asthma assessments and care from a study clinician, as well as asthma education including select environmental control measures. The participant's asthma may or may not improve while in this study.

2 Study Objectives

2.1 Primary Objective

The primary objective of the continuation of URECA to age 14/16 is to determine the wheezing, asthma and atopy phenotypes in minority children growing up in poor urban neighborhoods as they develop from birth through adolescence.

2.2 Secondary Objectives

Secondary objectives are:

1. To longitudinally analyze the development of lung function and its relationship to asthma.
2. To identify which urban exposures (allergens, pollutants, microbes, viral infections, stress) from early life onward affect the development and natural history through adolescence of asthma, allergic rhinitis and allergic sensitization.

3. To determine the association of pre-pubertal obesity and the effect of pubertal changes in adiposity and sex hormones on lung growth and asthma onset, prevalence and morbidity.

2.3 Exploratory Objectives

URECA has several exploratory objectives to use data and samples collected from the study participants:

1. To provide samples for analysis and outcomes data for use in mechanistic studies to understand relationships between environmental exposures, changes in immune development, and the development of allergic sensitization, allergic rhinitis and asthma (see Section 3.2.4).
2. To work collaboratively with investigators outside of the ICAC network on mechanistic or clinical studies to test novel hypotheses and to validate, compare and contrast findings from URECA with those in other cohorts or clinical studies.

3 Study Design

3.1 Description of the Study Design

The continuation protocol retains many of the successful features of the ongoing protocol; children will continue to be monitored through quarterly phone calls and yearly clinic visits (see Appendix B). Interim goals are to maintain a high level of participation in study procedures, and retain the current subjects through age 14-16 years. The quarterly calls serve a dual purpose in providing frequent assessments of respiratory symptoms, while also enabling staff to maintain close contact with the families to promote retention and adherence with study procedures. We will continue to conduct environmental sampling, immunologic profiling, and studies of body composition and changes in sex hormones and adipokines during puberty that will enable a longitudinal analytic approach.

We anticipate that the eventual expression of asthma will continue to evolve between ages 10 and 16 years, and thus, this stage of URECA will collect data on not only the development and resolution of asthma but also asthma morbidity and intensification of asthma severity. Finally, in school-aged children, we will be able to identify risk factors for the onset of allergic rhinitis and the transition in some children from allergic rhinitis to allergic asthma.

Study Endpoints

3.1.1 Primary Endpoint

The primary outcome of URECA through age 7 was the development of asthma, which was determined using an algorithm that evaluated reported physician diagnosis, lung function, symptoms, healthcare utilization and medication use (Table 1). Preliminary analyses of age 7 data indicate that approximately 29% of the URECA study participants met this definition for asthma.

For this continuing phase of URECA, we propose to further define asthma phenotypes based on the findings in ICAC-19 (APIC: Asthma Phenotypes in the Inner City) and the comprehensive and longitudinal observations conducted in URECA.

Data regarding asthma diagnosis, the frequency and severity of cough and wheezing illnesses, health care

utilization, and medication use will be collected during each of the quarterly telephone calls. Examinations will also be conducted as part of the scheduled study visits through age 16 years, and questions about wheezing and asthma will be asked at these visits as well. All of this information will be recorded into a centralized database for analysis.

3.1.2 Secondary Endpoints

A number of secondary outcome variables will also be assessed as follows:

1. Methacholine responsiveness – this will be ascertained at the 14-year visit.
2. Pulmonary function – pre- and post- bronchodilator spirometry and impulse oscillometry will be measured at each annual clinic visit, except for at age 14, unless the participant is unable to complete the methacholine challenge above.
3. Allergic sensitization – defined as a dichotomous variable (aeroallergen-specific IgE by skin test or serum test) or as a continuum (number of positive skin or sIgE tests, sum of sIgE values).
4. Allergic rhinitis – chronic seasonal or perennial rhinitis and corresponding allergen-specific IgE or skin test.

4 Study Population

Table 1. URECA Definition for Current Asthma = **A OR B OR C**

- A.** Doctor-diagnosed asthma: Doctor diagnosis of asthma at any time in life
And at least 1 of the following conditions in the last 12 months:
1. 1 or more wheezing episodes, **or**
 2. 1 or more doctor or emergency room visits for wheezing or asthma, **or**
 3. 1 or more hospitalizations for wheezing or asthma, **or**
 4. Use of controller medication for at least 6 months in the previous 12 months
- B.** Asthma diagnosis based on lung function:
Positive bronchial challenge (methacholine PC₂₀ ≤ 4 mg/ml) **or** reversibility ≥ 10%
And at least 1 of the following conditions in the last 12 months:
1. 1 or more wheezing episodes, **or**
 2. 1 or more doctor or emergency room visits for wheezing or asthma, **or**
 3. 1 or more hospitalizations for wheezing or asthma, **or**
 4. Use of controller medication for at least 6 months in the previous 12 months
- C.** Asthma diagnoses based on symptoms or health care utilization
At least 1 of the following conditions in the last 12 months:
1. 2 or more wheezing episodes, **or**
 2. 2 or more doctor or emergency room visits for wheezing or asthma, **or**
 3. 1 or more hospitalizations for wheezing or asthma, **or**
 4. Use of controller medication for at least 6 months in the previous 12 months

4.1 Population Description

To address the primary objectives of the URECA IV protocol, all the participants active in the URECA III protocol at age 10 will be invited to continue under the new protocol until age 14-16.

The URECA study is being conducted at the Johns Hopkins University School of Medicine, Baltimore, MD; Boston University School of Medicine, Boston, MA; Children’s Hospital of New York-Presbyterian, New York, NY; and Washington University School of Medicine, St. Louis, MO. At the time of enrollment, study participants had to live in the inner-city, defined for this study as specific contiguous neighborhoods where at least 20% of the population is below the federal poverty level. The resulting study populations in Baltimore and St. Louis are predominantly African-American. The populations in Boston and New York are more evenly divided between African-American and Hispanic (Table 2).

Table 2. URECA Study Population by Ethnicity and Race of Child and Site

	Hispanic	African-American	Mixed Race	White, Asian, Other
Baltimore	2%	90%	4%	4%
Boston	31%	54%	11%	4%
New York	61%	36%	3%	0%
St. Louis	2%	88%	8%	1%
Total	21%	70%	7%	3%

The URECA study enrolled 560 inner-city children at high risk for developing allergic diseases and asthma on the basis of a parental history of asthma, allergic rhinitis or atopic dermatitis. A small comparison sample of 49 babies without allergic family history was also enrolled. Babies born at 34 weeks gestation or later were allowed into the study, provided that there was no significant respiratory distress as defined by the exclusion criteria, and they were otherwise healthy. Infants with other lung diseases such as respiratory distress syndrome, bronchopulmonary dysplasia and pneumonias were not enrolled due to confounding effects on respiratory symptoms and lung function. Babies of HIV-infected mothers were excluded due to immunomodulatory effects of HIV infection, and of anti-retroviral medications such as zidovudine (AZT).

4.2 Participant Inclusion Criteria

Participants to be included in URECA IV are those who are active in the URECA III protocol at approximately age 10.

5 Study Procedures/Evaluations

5.1 Clinical Evaluations

If any of the assessments do not occur at a visit that assessment can be completed at the next visit. The study assessments and techniques employed in the original URECA protocol will continue to be followed as described below.

5.1.1 Dust Analysis for Allergen and Microbial Products

Dust specimens will be collected at approximately ages 12, 14, and 16 following procedures described in the URECA IV MOP for ICAC-07. One combined dust sample will be collected from the child's bed (defined as the location the child sleeps the most) and bedroom floor. Handling of specimens and laboratory assays will remain the same as in the original protocol.

5.1.2 Allergen Skin Testing

Skin testing to a panel of aeroallergens will be performed at 14 years of age. These will include a panel of well-characterized antigens that have been associated with respiratory allergies and asthma in this age group. Only personnel that are trained in the procedures established by the URECA IV MOP for ICAC-07 will perform this test. Skin testing will be done by the prick technique using the GreerPick system (Greer; Lenoir, NC) in accordance with generally accepted guidelines.⁷ Tests will be read after 15 minutes by measuring the wheal for each antigen and for the controls. Participants will be asked to stop taking antihistamines for a period of time specified in the URECA IV MOP prior to the test to limit interference with the results of the skin test.

5.1.3 Pre/Post Bronchodilator Pulmonary Function Testing

Annual study visits, except at age 14, will include pre/post bronchodilator testing. After completion of spirometry as described in the main protocol, albuterol via MDI with spacer will be administered. Fifteen minutes later the spirometry and IOS will be repeated. Participants who fail to meet the criteria for methacholine challenge testing will complete IOS and spirometry reversibility at the 14-year clinic visit.

5.1.4 Methacholine Challenge

The 14-year clinic visit will include a methacholine challenge test. Airway responsiveness will be measured by assessing the dose of methacholine required to produce a drop in FEV₁ of 20% (PD₂₀) after the administration of increasing concentrations of methacholine using the small volume nebulizer-tidal breathing technique. An ICAC trained and certified pulmonary function technician will perform the test based on the procedures outlined in the URECA IV MOP for ICAC-07. These procedures will be used in ICAC due to the documented safety of the approach in large pediatric asthma populations. Provocholine® will be used as the commercial source of methacholine, since it is an FDA approved product for children as young as 5 years of age. Testing criteria are described in the MOP for ICAC-07 that ensure the quality of the data collected and the safety of the study participants.

5.1.5 Exhaled Nitric Oxide Measurement

Measurement of eNO will be obtained at the 12-, 14-, and 16-year clinic visits, and will be measured prior to the measurement of spirometry. Exhaled NO will be measured employing a technique modified after Silkoff et al.⁸ and following American Thoracic Society (ATS) guidelines for eNO assessment.⁹ Nitric oxide concentrations will be measured using a commercially-available analyzer and a procedure described in detail in the URECA IV MOP for ICAC-07.

5.1.6 Induced Sputum

Sputum will be induced by inhalation of hypertonic saline solution using the method that was used in the Asthma Clinical Research Network (ACRN).¹⁰ Safety monitoring per spirometry and symptom report will be performed during and after sputum induction. Processing will be performed according to the URECA IV MOP for ICAC-07. Slides will be read at a central site for cellular determinations. Residual sputum cells will be processed to analyze cell markers and RNA and DNA isolation for expression and epigenetic studies. Sputum supernatants will be collected, aliquoted, and stored pending further analysis.

5.1.7 Nasal Epithelial Cell Collection

Nasal epithelial cell samples will be obtained by using a cytology brush. A trained clinician will conduct the procedure. The inferior turbinate of one nasal passage will be sampled to obtain an adequate number of epithelial cells for mRNA and DNA isolation. The sampled area will be observed for hemostasis. Procedural details and instructions for processing the samples are included in the URECA IV MOP for ICAC-07.

5.1.8 Fitness Testing

The Six-Minute Walk Test will be used at the 11-, 13-, and 15-year clinic visits to assess the child's level of fitness.¹¹ For this test the child is asked to walk around two cones placed in a hallway 60 meters apart at a fast pace for six minutes. The number of meters walked is measured, as well as the child's pulse and respiratory rate. The child is asked to rate his or her level of fatigue on a visual analogue scale.

5.2 Laboratory Evaluations

5.2.1 Clinical and Research Laboratory Evaluations and Specimen Collection

5.2.1.1 Urine Pregnancy Testing

All females who have reached menarche will be required to have a urine pregnancy test prior to any procedures that entail any risk, but not prior to a blood draw. Results of all pregnancy tests will be given to the participant and/or caretaker following state laws.

Pregnancy in girls will not result in withdrawal from the study, but will be reported on the appropriate CRF and followed to outcome. If the pregnancy results in anything other than a normal birth or elective abortion of a healthy fetus, it will be reported as a SAE.

5.2.1.2 Urine Collection

Urine will be collected at the 12-, 14-, and 16-year clinic visits for measurement of urinary cotinine, a marker for tobacco smoke exposure. A urine sample will be collected following procedures described in the URECA IV MOP for ICAC-07.

5.2.1.3 Blood Collection

Blood will be collected while participants are active in URECA IV to measure serum markers of adiposity and systemic inflammation (e.g., leptin, adiponectin, hsCRP, IL-6, TNF- α), levels of sex hormones (e.g. estrogen, testosterone), and insulin resistance (e.g. fasting insulin and glucose), as well as total and allergen-specific IgE. Blood is also used for analysis of immunoregulatory mRNA and protein responses as a measurement of individual and developmental immunologic patterns. Plasma and serum proteins will also be analyzed, and these assays will be performed in a multiplex format whenever possible due to the limited availability of blood and plasma from child subjects. In addition, systems approaches (e.g. microarray, proteomics, epigenetics) will also be used in the analysis as these technologies are developed to assess global patterns in immunoregulatory factors in the blood or in blood cells. Additional analysis may be done for potential biomarkers related to asthma and allergies.

The amount of blood drawn will follow the NIH Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center (5/2012). For pediatric patients, no more than 5 mL/kg may be drawn for research purposes in a single day, and no more than 9.5 mL/kg may be drawn over any eight-week period. For adults the amount shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

5.2.1.4 Nasal Fluid

Nasal fluids will be analyzed for proteins (e.g. cytokines) using methods as listed above.

5.2.1.5 Immunologic Studies

The overall goal of the immunologic studies is to define effects of environmental stimuli on immune development, and then identify patterns of immune development that are associated with the development of asthma. These studies will be conducted using cells and fluids from blood, the upper airway, and possibly urine.

5.2.2 Specimen Preparation, Handling and Shipping

5.2.2.1 Instructions for Specimen Storage

Most samples that are collected at the clinical sites will be shipped directly to processing laboratories. However, samples that will need to be stored for at least 3 months (specimens of DNA, plasma, etc.), as well as specimens that are generated by laboratories (e.g. supernatant fluids from cytokine secretion assays) that are awaiting further analysis, will be sent to a central storage facility pending analysis. This facility will maintain a computerized inventory of all of the specimens to be analyzed, and will ship the specimens

to designated laboratories for analysis upon request. In order to protect subjects, all samples are stored and shipped under code so that neither the child nor his/her parents can be identified individually.

5.2.2.2 Specimen Shipment

Instructions for sample preparation, handling, storage, and shipping are included in the URECA IV MOP for ICAC-07. Principal Investigators will be responsible for knowing about and observing all the regulations for classification, packaging and labeling, permits or authorizations, and personnel training for shipment of biological and hazardous materials required for the conduct of this study.

5.3 Substudies

URECA also provides an outstanding opportunity to conduct studies of mechanisms related to the onset, progression and prevention of allergic rhinitis and asthma, and we have designed extensive collaborative studies with the three proposed ICAC Mechanistic Study Centers, including:

1. Studies to define mechanisms of microbial effects on the risk of allergic diseases and asthma (collaboration with the University of California San Francisco),
2. A systems-based analysis to integrate environmental exposures, epigenetic changes via methylation, gene transcription, immune development and asthma/allergy clinical outcomes and biomarkers (collaboration with the Benaroya Research Institute, Seattle, WA), and
3. Analysis of epitope recognition and functional characteristics of cockroach-specific T cells, and relationship to cockroach exposure, sensitization, and expression of clinical disease (collaboration with the LaJolla Institute of Allergy and Immunology).

Each substudy or ancillary study involving URECA participants or samples from participants will be accompanied by a proposal specifying the objectives of the substudy, the sample requirements, and the analytic plan. If additional samples or procedures not included in the main study consent are required for the substudy, an informed consent addendum will need to be signed by the participant's caretaker/guardian, and the participant will sign an assent form.

6 Research Use of Stored Human Samples, Specimens or Data

6.1 Use of Stored Samples/Data

Extra research samples remaining once the specified analyses are conducted will be stored long-term for future research in the field of asthma. Participants will be asked to give permission for long-term storage and future use during the consent process.

6.2 Disposition of Stored Samples/Data

Stored samples will be maintained in long-term storage until they are used or until permission is obtained from the ICAC Steering Committee and DAIT/NIH to destroy them.

7 Study Schedule

7.1 Informed Consent

Once the URECA IV protocol is approved, informed consent to continue participation will be obtained by the URECA staff at the earliest time that the child's parent/guardian is seen in person. If the family has not been seen in person and the child reaches 10 years of age, a telephone consenting procedure will be used to continue with quarterly phone calls, according to IRB requirements. In these cases, written consent will be obtained before the next clinic visit. We will also obtain written assent from participating children according to local IRB guidelines before enrollment into URECA IV.

7.2 11-Year Clinic Visit

At the 11-year clinic visit, a brief physical examination will be conducted, to focus on ears, nose, throat, chest and skin, and including Tanner staging. BIA will be performed to assess adiposity. Pulmonary function measurements (spirometry, impulse oscillation spirometry) will be performed, with pre- and post-bronchodilator measurements. In addition, a sample of nasal epithelial cells will be collected. The Six-Minute Walk Test will be performed to assess fitness.

7.3 12-Year Clinic Visit

At the 12-year clinic visit, a brief physical examination will be conducted, to focus on ears, nose, throat, chest and skin, and including Tanner staging, and a urine sample will be collected for cotinine measurement. Blood and urine specimens will also be collected at this visit. Spirometry and impulse oscillation spirometry with reversibility, and measurement of exhaled nitric oxide (eNO) will be performed. The questionnaire set will include information about levels of personal and household stress.

7.4 13-Year Clinic Visit

At the 13-year clinic visit, a brief physical examination will be conducted, to focus on ears, nose, throat, chest and skin, and including Tanner staging. BIA will be performed to assess adiposity. Pulmonary function measurements (spirometry, impulse oscillation spirometry) will be performed, with pre- and post-bronchodilator measurements. The Six-Minute Walk Test will be performed to assess fitness.

7.5 14-Year Clinic Visits

At the 14-year clinic visit, a brief physical examination will be conducted, to focus on ears, nose, throat, chest and skin, and including Tanner staging. The pulmonary function measurements to be performed at this visit will include baseline spirometry and IOS, a methacholine challenge test, and measurement of exhaled nitric oxide (eNO). Spirometry and impulse oscillation spirometry with reversibility will be performed on those children who do not meet the criteria for performing the methacholine challenge. Blood and urine specimens will also be collected at this visit. Skin testing for indoor and outdoor allergens will be repeated. A nasopharyngeal mucus specimen will be obtained from the child. The questionnaire set will include information about levels of personal and household stress.

7.6 15-Year Clinic Visit

At the 15-year clinic visit, a brief physical examination will be conducted, to focus on ears, nose, throat, chest and skin, and including Tanner staging. BIA will be performed to assess adiposity. Pulmonary function measurements (spirometry, impulse oscillation spirometry) will be performed, with pre- and post-bronchodilator measurements. In addition, the Six-Minute Walk Test will be performed to assess fitness.

7.7 16-Year Clinic Visit

At the 16-year clinic visit, a brief physical examination will be conducted, to focus on ears, nose, throat, chest and skin, and including Tanner staging, and urine and blood samples will be collected. Spirometry and impulse oscillation spirometry with reversibility, and measurement of exhaled nitric oxide will be performed. The questionnaire set will include information about levels of personal and household stress.

7.8 Induced Sputum Visit

Induced sputum will be performed at a separate visit which can occur at any time at or between the 14- and 16-year clinic visit. This will be accompanied by collection of nasal epithelial cells (brushing and cells in secretions) and fluid.

7.9 Quarterly Phone Calls

Phone calls will be placed to the family every 3 months between clinic visits to collect data related to study outcomes, and also to continue to engage the family in the study and provide informational updates. Questionnaires to be administered will collect data on respiratory symptoms, environmental exposures and diet, health care utilization, and medication use. These phone calls will continue many questionnaires from the original URECA study in addition to new questions appropriate for the older age group.

7.10 Home Environmental Exposure Evaluation

At the 12-, 14-, and 16-year clinic visits, the family will be given a dust collection kit, which includes instructions on how to collect a dust sample (refer to the URECA IV MOP for ICAC-07 for Dust Collector Instruction Card). A combined dust sample from the child's bedroom floor and the child's bed will be collected. The room where the child sleeps most nights will be considered the bedroom. Measuring templates will be used to delineate the areas to be vacuumed. Dust will be collected using a vacuum cleaner with a special dust collection filter attached. The dust collector will be placed into a sealable plastic bag and mailed back to the study center for temporary storage (frozen). Crude samples will be batched and shipped to a central laboratory by express mail for sieving, extraction, and analysis. The dust specimens will be assayed to measure the concentration allergens such as: Der p 1, Der f 1, Bla g 1, Bla g 2, Fel d 1, Can f 1, Alt a 1, and Mus m 1. Additional allergens of interest and markers of fungal and microbial exposure may be measured. In addition, the caretaker will complete a dust collection questionnaire, which will be mailed back with the dust collector.

When problems arise with the family doing the environmental sample collections, staff may go to the home to collect the dust samples.

7.11 Additional Visits or Contacts

Additional visits or telephone contacts may be made for any of the following reasons:

1. A visit was conducted but 1 or more planned procedures were not completed, or a result was not obtained.
2. Contact information needs to be updated (no more often than every 6 months).

Any of the assessments listed above may be conducted at additional visits or by telephone (as appropriate)—either for the first time or for reassessment.

During the consent process, the participants will be asked to consent or assent to the possibility of additional visits or contacts as described in this section (Section 7.10). No participant will be subjected to an assessment for which he or she has not provided written consent or assent, and no informed consent/assent form will contain any information that has not been approved by the IRB of record.

7.12 Recontact of Subjects after Study Termination

The caretaker/guardian of the child participant is asked during the informed consent process to indicate that contact for future research studies is permissible.

8 Assessment of Safety

8.1 Definition of an Adverse Event (AE)

The URECA study has been designated as an observational birth cohort study by the ICAC and DAIT. As such, this study involves minimal active intervention outside of the normal standard of care for the participant. No study drugs are used in the URECA study. Adverse events are limited to any occurrence or worsening of an undesirable or unintended sign (including an abnormal laboratory finding), symptom, or disease in a study subject that is specifically associated (probably, possibly, or definitely – defined in section 8.4.1.2) with a study procedure that is not part of the normal standard of care for the participant. In the URECA study, adverse events are those related to the blood draws, nasal sample collection, allergen skin testing, sputum collection, or pulmonary function tests not included as part of standard of care for this group of patients.

8.2 Definition of a Serious Adverse Event (SAE)

A Serious Adverse Event (SAE) is defined as an AE resulting in one of the following outcomes:

- Death during the period of protocol-defined surveillance
- Life Threatening Event (defined as an event that places a participant at immediate risk of death)
- Inpatient hospitalization or prolongation of existing hospitalization during the period of protocol-defined surveillance
- Congenital anomaly or birth defect
- Persistent or significant disability/incapacity

Any other condition which, in the judgment of the investigator, represents a significant hazard, such as an important medical event that does not result in one of the above outcomes, may be considered a serious adverse event when the event jeopardizes the participant or requires medical or surgical intervention to prevent one of the outcomes listed above.

8.3 Death Not Related to Study Procedures

A death occurring in a study participant not associated with blood draws, nasal sample collection, allergen skin testing, sputum collection, or pulmonary function tests will be reported as a serious adverse event not related to study procedures. The reporting process will follow the SAE reporting process.

8.4 Methods and Timing for Assessing, Recording, Analyzing and Adverse Events

8.4.1 Methods and Timing for Assessment

8.4.1.1 Grading of Adverse Events/Serious Adverse Events

Each adverse event will be assessed for severity and classified into one the categories below:

- **Grade 1 (Mild):** Event requires minimal or no treatment and do not interfere with the participant's daily activities.
- **Grade 2 (Moderate):** Event results in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Grade 3 (Severe):** Event interrupts a subject's usual daily activity or functioning and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- **Grade 4 (Life threatening):** Any adverse drug experience that places the participant, in the view of the investigator, at immediate risk of death from the event as it occurred.
- **Grade 5 (Death)**

8.4.1.2 Attribution of Adverse Event to Study Procedure

For the purpose of this study, only AEs related to a study procedure (Section 8.1) will be reportable and by definition will always be assessed as related. The degree of certainty about relatedness will be graded using the 2 categories below.

- **Definitely Related:** There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs within a reasonable timeframe after study procedure(s) and cannot be explained by concurrent disease or other drugs or chemicals.
- **Possibly Related:** There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after study procedure(s)). However, the influence of other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events). Although an adverse event may be judged only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "definitely related".

8.4.2 Recording/Documentation

Adverse events and serious adverse events related to the study procedures will be recorded on an appropriate eCRF. The event, the procedure to which it is related, start date, stop date, and severity of each reportable event will be recorded on the eCRF.

8.4.3 Analysis/Management

The rate of AEs has historically been low in this study; therefore, the primary analysis of AEs will occur by the medical monitor as described in Section 8.5. Additional management is not expected to be necessary.

8.5 Reporting Procedures

All adverse events related to the blood draws, nasal sample collection, allergen skin testing, sputum collection, or pulmonary function tests occurring during the study will be reported to the SACCC. The SACCC reports to the NIAID Medical Monitor who is responsible for passing the information on to the NIAID Safety Monitoring Committee (SMCDSMB). Adverse events will be followed until resolved or considered stable.

8.5.1 Serious Adverse Event Reporting

Adverse event reporting requirements to the NIAID DSMB for this protocol are as follows:

- Investigators will submit a completed serious adverse event report to the NIAID DSMB within 7 days after becoming aware of a subject death, a potentially life-threatening (Grade 4) serious adverse event that is possibly or definitely related to study procedure(s), an urgent inpatient hospitalization or transfer to the ICU.
- Investigators will submit a completed serious adverse event report to the NIAID DSMB within 15 days after becoming aware of any Grade 3 (severe) adverse event that is possibly or definitely related to study procedure(s), or an inpatient hospitalization (other than elective), a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.
- Investigators will report within 15 days on any other event or condition regardless of grade, which in their judgment represents an event reportable to the NIAID DSMB.
- Investigators will forward all safety reports and related communications to the NIAID DSMB within 15 days of receipt.
- A summary of all adverse events will be reported to the NIAID DSMB with a continuing review submission.

8.5.2 Adverse Event Reporting

Adverse events which are not SAEs or Grade 2 or higher unexpected AEs, will be recorded on the appropriate case report form and sent to the SACCC for incorporation into annual reporting to the NIAID and DSMB.

8.5.3 Reporting to the IRB

The Principal Investigator (or delegate) must report adverse events and serious adverse events to the central IRB promptly in accordance with local regulations or policies, in addition to providing the information to the SACCC.

8.5.4 Reporting Pregnancy

A pregnancy will not be reported as an adverse event for follow-up purposes. All pregnancies that are identified during the study will be followed to conclusion and the outcome of each will be reported. If the pregnancy results in anything other than a normal birth or elective abortion of a healthy fetus, it will be reported as a serious adverse event.

8.6 Type and Duration of the Follow-up of Participants after Adverse Events

The site investigator must apply his or her clinical judgment as to whether an AE is of sufficient severity to require that the participant immediately be removed from the study. The investigator must institute any necessary medical therapy to protect a participant from any immediate dangers.

An AE will be followed until any of the following takes place: a) it is resolved, b) participant is stable, or c) a minimum of 30 days after participant is discontinued from the study, whichever comes first.

8.7 Participant Discontinuation

Participants may choose to withdraw from the study at any time, during a study visit, or afterwards in person, by telephone, or in writing.

Participants may be prematurely terminated from the study for the following reasons:

1. The participant elects to withdraw consent from all future study activities.
2. The participant is “lost to follow-up” (i.e., no further follow-up is possible because attempts to re-establish contact with the participant have failed).
3. The investigator no longer believes participation is in the best interest of the participant.

Participants will be notified, if possible, if they are discontinued from the study. No additional follow-up will be required.

8.8 Replacement of a Participant Who Discontinues Study Participation

URECA study participants will not be replaced after discontinuation. However, “lost” study participants who are subsequently relocated and who provide informed consent, may be re-activated.

9 Clinical Monitoring Structure

9.1 Site Monitoring Plan

Clinical site monitoring will be conducted according to the URECA Clinical Monitoring Plan (CMP) for ICAC-07 to ensure that human subject protection, study procedures, and laboratory and data collection processes are of high quality and meet sponsor, International Conference on Harmonisation, Good Clinical Practice, and regulatory guidelines. Representatives from the National Institute of Allergy and Infectious Diseases (NIAID) and/or the SACCC will visit each clinical site or meet with each clinical site via telephone during a specified timeframe according to the URECA CMP for ICAC-07. Key study personnel must be available to assist the visitors during these visits or attend the call if completed via telephone. Additional details regarding clinical site monitoring, including remote monitoring, are outlined in the URECA CMP and MOP for ICAC-07.

9.2 Safety Monitoring

Safety monitoring will be performed following the URECA Safety Management Plan (SMP).

9.2.1 Medical Monitor Review

The DAIT Medical Monitor will review all serious adverse events and Grade 2 or higher unexpected adverse events immediately upon notification by the SACCC. The Medical Monitor will review reports prepared by the SACCC of all adverse events at least quarterly.

9.2.2 DSMB Review

The DSMB will review any events as requested by the Investigators, SACCC, or DAIT Medical Monitor. They will review a listing of all adverse events once per year. Further, the DSMB will be informed of expedited SAEs at the same time as the central IRB.

10 Statistical Considerations

10.1 Overview

The URECA extension until the age of 14-16 years has the following specific aims that will be accomplished under this protocol. Mechanistic and substudy aims will be addressed in separate analytic plans.

1. To determine the wheezing, asthma and atopy phenotypes in minority children growing up in poor urban neighborhoods as they develop from birth through adolescence.
2. To longitudinally analyze the development of lung function and its relationship to asthma.

3. To identify which urban exposures (allergens, pollutants, microbes, viral infections, stress) from early life onward affect the development and natural history through adolescence of asthma, allergic rhinitis and allergic sensitization.
4. To determine the association of pre-pubertal obesity and the effect of pubertal changes in adiposity and sex hormones on lung growth and asthma onset, prevalence and morbidity.

10.2 Endpoints

10.2.1 Primary Endpoints

The primary endpoints for this phase of the URECA study are wheezing, asthma and atopy phenotypes in minority children.

10.2.2 Secondary Endpoints

Secondary endpoints include:

1. Methacholine responsiveness – this will be ascertained at the 14-year visit.
2. Pulmonary function – pre- and post- bronchodilator spirometry and impulse oscillometry will be measured at each annual clinic visit, except for at age 14, unless the participant is unable to complete the methacholine challenge above.
3. Allergic sensitization – defined as a dichotomous variable (aeroallergen-specific IgE by skin test or serum test) or as a continuum (number of positive skin or sIgE tests, sum of sIgE values).
4. Allergic rhinitis – chronic seasonal or perennial rhinitis and corresponding allergen-specific IgE or skin test.

10.3 Measures to Minimize Bias

The primary threat to validity in a cohort study such as URECA is missing data due to loss-to-follow-up or missed visits and procedures. The URECA study to date has done a remarkable job of retaining the participants, with 78% of the original cohort still active at age 8/9. In the second two phases of URECA, 95% of expected visits have been completed.

Missing data create problems because fewer observations will reduce the power of the study. If the data are nonrandomly missing, i.e. associated with either the predictor or the outcome, estimates generated in the analyses may be biased, possibly leading to invalid inferences. For example, children with asthma symptoms might miss their spirometry measurement. This can lead to nonrandomly missing data and can complicate analyses.

The pattern of missing data will be explored for every analysis. Missing data can generally be characterized as being in 1 of 3 classes--missing completely at random, missing at random, and not missing at random (also called nonignorably missing or informatively

missing).¹²⁻¹⁴ If data are missing completely at random or missing at random, we will select the most appropriate available statistical method, typically a linear or generalized linear model or survival model that addresses the specific question. If the data are informatively missing, selection of the appropriate technique will require further consideration. Currently the statistical community has not reached a consensus on how to best address the issue of informatively missing data, but many approaches have been proposed.¹³⁻¹⁹ Analyses conducted by Rho statisticians for multiple projects suggest in many cases the best approach is to use a linear mixed model even in the face of informatively missing data.²⁰ URECA analyses to date have shown most missing data to be the result of missed visits or sample analysis problems, and not informatively missing.

10.4 Analysis Plan

10.4.1 Analysis Population

10.4.2 Primary Analysis of Primary Objective

The purpose of the primary analysis in the fourth phase of URECA will be to identify wheezing and asthma phenotypes in the children through the age of 14 to 16 years. Furthermore, we will identify risk factors for the asthma phenotypes related to environmental exposures, immune responses and biomarkers, weight and fitness status, and airway and environmental microbiota.

Using the extensively and longitudinally characterized URECA cohort, we will:

1. Categorize URECA subjects into already described early life patterns of wheezing (Tucson Children's Respiratory Study, Avon Longitudinal Study of Parents and Children, Prevention and Incidence of Asthma and Mite Allergy) and development of atopic disorders (Manchester) to determine if these patterns are indeed observed among inner-city American children.
2. Evaluate if the phenotypes of easy-to-treat and difficult-to-treat asthma among inner-city children developed in APIC (ICAC-19: Asthma Phenotypes in the Inner City) are reflected by the URECA cohort. APIC was designed to determine, by an extensive and longitudinal data base collection, the phenotypic characteristics associated with easy-to-treat versus difficult-to-treat asthma in inner city children. The subjects (n=619) had individual characteristics collected, including allergic sensitization, rhinitis, pulmonary function (including spirometry with reversibility, lung volumes, methacholine responsiveness), environmental exposures (including stress), immune biomarkers (e.g. steroid responsiveness, sputum biomarkers) and diet. The results of this study will provide new insight into asthma phenotypes in inner city children, including children with difficult-to-treat asthma. The phenotypes will be established in APIC, and the risk factors and evolution of these phenotypes will be determined in URECA.

3. Using longitudinal data obtained from the URECA cohort through the age of 14-16 years, we will perform a latent class analysis to identify patterns of wheezing, asthma and development of atopy (allergic sensitization) over time. Latent class analysis (LCA) represents a broad class of random coefficient methods that summarize meaningful subgroups of participants based on their patterns of response profiles. LCA has been used to define unique classes of individuals based on wheeze, patterns of atopy and pulmonary function. As noted above, this approach has become an important alternative to traditional variable-centered methods that can produce summaries of how one variable relates to another but provides little information as to how individuals cluster with regard to their response profiles. Latent class analysis postulates the existence of unobserved categorical variables that divide the population of interest into classes; members of the population with a set of observed variables will respond differently depending on the latent class to which they belong. In URECA the comprehensive longitudinal measurements within a child allow the application of latent class analysis where periods have the greatest impact on the development and maintenance of asthma through the teen years. In particular, symptoms, exacerbations, allergy, immunologic measures, BMI, fitness, psychosocial factors and objective measurements of lung function, spirometry, eNO and IOS will define clusters of asthma phenotypes based on a combination of these variables.

10.4.3 Analysis of Secondary Objectives

10.4.3.1 Lung function

Similar methods to those described above will be used to examine predictors of differences in lung function parameters and of changes in lung function during pubertal development. In addition, the relationship of lung function parameters to the natural history of asthma and other secondary outcomes will be explored. The lung function parameters to be included in these analyses are:

1. Spirometry reversibility – with albuterol, and at age 13 with ipratropium
2. Impulse oscillometry (IOS)
3. PD₂₀ as measured by methacholine challenge

10.4.3.2 Urban exposures related to asthma, allergic sensitivity and allergic rhinitis

Logistic regression will be used to identify relationships between airway and blood immune responses or biomarkers and the presence or absence of asthma or specific asthma phenotypes. The model will include immunologic explanatory variables that describe “cytokine dysfunction” and “patterns of cytokine responses”. The explanatory variables, developed during the exploratory analysis phases, will be clearly specified prior to the analysis and may include composite summary scores to describe inflammation or immunologic functions at different time points, individual cytokine levels of unique importance (for cytokines that are independent of composite summary scores), and/or variables for at-risk subgroups defined by, for example, temporal occurrence of immune

system developmental events or evidence of cytokine dysfunction. Explanatory variables will be selected for inclusion in the model based on potential importance noted in the literature and hypotheses generated during exploratory analyses. The resulting regression model will provide insight into how timing, type, and sequence of immunologic developments (defined by cytokine responses) impact the risk of developing asthma by age 14.

Similar approaches will be used to identify patterns of immunologic development associated with development of atopy and allergic rhinitis. One analysis will be analogous to that described for the primary analysis using a logistic regression to model the presence or absence of atopy or allergic rhinitis as a function of immunologic explanatory variables describing “cytokine dysfunction” and “patterns of cytokine responses”. Additional analyses using linear regression or survival analysis methods will examine the relationship between the age of onset of atopic sensitization (assessed using allergen-specific IgE levels) and immunologic explanatory variables.

In addition, we will collaborate with the Benaroya Research Institute to conduct a systems-based analysis of environmental influences, epigenetic changes, immune development, and outcomes as listed above. A detailed analysis plan will be available in the ancillary study protocol.

10.4.3.3 Effect of pubertal changes in adiposity and sex hormones on lung growth and asthma onset, prevalence and morbidity

Levels of steroid hormones, steroid binding proteins, leptin, adiponectin and serum inflammatory indicators (IL-6, TNF- α , CRP) at 8, 10, 12 and 14, and 16 years of age will be compared to asthma status by ANOVA. These analyses will also be stratified by sex, and we will test whether sex hormones and sex hormone binding proteins modify the relationship between leptin and asthma prevalence in post-pubertal girls and boys. Body habitus and measurements of fitness (6-minute walk in clinic) will also be compared among children with vs. without asthma. We expect that children at greatest risk for asthma will be those with low fitness and activity, high BMI, increased serum inflammatory markers, and atopy.

We will use trajectory analysis to examine whether changes in weight or BMI are related to asthma prevalence in children between the ages of 10 and 14 years. We will use semiparametric mixture modeling—which combines latent growth curve and mixture modeling—to identify growth trajectories over time separately for boys and girls. Trajectory parameters will be estimated using the maximum likelihood approach built upon a binary logit model, using the BIC (Bayesian information criterion) to identify the best model. The objective of model selection is to summarize the distinctive features as parsimoniously as possible. The resulting growth trajectories will be used as predictor variables in a logistic regression model predicting development of asthma. The model will be tested for effect modification by sex.

10.4.4 Analysis of Exploratory Endpoints

Exploratory analyses for mechanistic objectives will be described in the analytic plan accompanying the exploratory or substudy proposal.

10.5 Sample Size Considerations

The assumptions for our original power calculation were conservative; it appears we will exceed our estimated power due to 1) over-enrollment at the start of the study, 2) a lower dropout rate than anticipated, and 3) a higher incidence of wheeze and asthma than expected.

We originally projected that we would need to enroll 500 children to have 350 children available for analyses at age 3; we eventually enrolled 560. Our original calculation assumed a 30% dropout rate by age 3; whereas the dropout rate at age 3 was actually 14% and by age 8 it is 22%, with only 50 8-year visits remaining. We assumed an incidence of recurrent wheeze of 17%, but observed a rate of 36%, over twice that upon which we based our calculations. For our age 7 power calculations, we used a 15% estimate for asthma incidence, but have observed 29%.

For our primary analysis of asthma, we were originally powered ($\geq 80\%$) to detect a relative risk of 2.0 for immune dysfunction (with an assumed prevalence of 25%). With a larger analysis population at age 7 of ~420 and an asthma incidence of 25%, our power to detect a relative risk of 2.0 is $\geq 97\%$, and we would have 80% power to detect an effect in the range of a 1.6 relative risk.

11 Quality Control and Quality Assurance

Training of study staff will be conducted prior to beginning any new procedures. All staff members will be required to complete certification and quality control in all applicable study procedures as outlined in the URECA IV MOP for ICAC-07. The site principal investigator and study coordinator(s) will be responsible for ensuring that all procedures are performed according to the protocol. Periodic reviews of procedures will be conducted by the study coordinator or other trained personnel according to an individual schedule for each staff member that is based on the activities he/she is responsible for conducting. Details of the quality control plan, including certifications and quality control of study procedures, are provided in the URECA IV MOP for ICAC-07.

12 Ethics/Protection of Human Subjects

12.1 The Belmont Report

In accordance with the [FWA00005897](#): “This institution assures that all of its activities related to human subject research, regardless of funding source, will be guided by the ethical principles of The Belmont Report.” Additionally, the investigator assures that all activities of

this protocol will be guided by the ethical principles of The Belmont Report, 45 CFR 46 and all of its subparts (A, B, C and D).

12.2 Institutional Review Board

A copy of the protocol, informed consent forms, other information to be completed by participants, such as survey instruments or questionnaires, and any proposed advertising/recruitment materials will be submitted to the IRB for written approval.

All subsequent amendments to the protocol, informed consent documents, and other study documentation referenced above must be reviewed and approved by the IRB before implementation. The annual Continuing Review must also be reviewed and approved by the IRB throughout the duration of the study.

The IRB will be notified of SAEs and protocol violations.

12.3 Informed Consent Process

The informed consent and assent forms are a means of providing information about the study to a prospective participant/guardian to allow for an informed decision about participation in the study. The parent or legal guardian of the participating child (or their legally acceptable representative) must read, sign, and date the informed consent form before continuing the study or undergoing any study-specific assessments. Consent materials for participants who do not speak or read English will be translated into Spanish for clinical sites with Spanish-speaking staff.

The informed consent and assent forms will be revised whenever important new safety information is available, whenever the protocol is amended with changes that require re-consent/re-assent by participants, and/or whenever any new information becomes available that may affect participation in the study.

A copy of the informed consent form (and assent form, if applicable) will be given to a prospective participant/caretaker for review. The prospective participant/caretaker will be told that being in the study is voluntary and that he or she may withdraw from the study at any time for any reason.

12.4 Assent Process

All URECA children will sign a written assent form as described above.

12.5 Participant Confidentiality

Following Health Insurance Portability and Accountability Act guidelines, a participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number and this number, rather than a name, will be used to collect, store, and report participant information. Data reported in medical journals

or scientific meetings will be presented in aggregate for participants as a whole. No individual participant will be identified in any way.

Participant confidentiality will be strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biologic samples in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to medical records (office, clinic, or hospital) for the participants in this study. The clinical site will permit access to such records.

12.6 Study Discontinuation

There are no study halting rules. However, the study may be discontinued at any time at the discretion of the NIH.

13 Data Handling and Record Keeping

Study data will be entered in a login-secured, web-based electronic data capture (EDC) system. The system operates on all Internet browser platforms and is designed to handle numerous simultaneous studies within a program. Physical, logical, and operational security related to the data is implemented by a network operation center. Additional details regarding the EDC system are provided in the Data Management Plan (DMP) for ICAC-07.

13.1 Data Management Responsibilities

The data management tasks required for this study are a joint responsibility of the SACCC and clinical site staff. The clinical site staff are responsible for collecting and entering study data into the EDC system per the protocol and specific guidelines in the URECA IV MOP for ICAC-07, for ensuring the accuracy of these data, and for maintaining and organizing all original source documents and any additional sources of data. The EDC system has been validated and is compliant with Part 11 Title 21 of the Code of Federal Regulations. The site principal investigator is responsible for supervising the data collection and data management processes at the clinical site to ensure the overall quality of the data generated by all clinical site staff. The SACCC is responsible for ensuring the quality of the data at all the clinical sites. The specific responsibilities of the clinical site staff, site principal investigators, and the SACCC are included in the DMP for ICAC-07. All data management activities at the SACCC and clinical sites will be conducted in accordance with the DMP.

13.2 Data Capture Methods

Data will be captured onto paper CRFs for later entry into eCRFs, or directly into eCRFs. The first recording of any information captured for the study will be considered the source document, which may be, but is not limited to, a medical record, a laboratory or clinical report, a paper CRF, or an eCRF.

The details regarding the electronic verification of all data fields, including univariate and multivariate validation (i.e., range checks and cross-field and cross-form checks), validation of data omission, and query management are listed in the DMP.

13.3 Types of Data

Clinical, demographic, laboratory, and AE data will be collected for this study.

13.4 Source Documents and Access to Source Data/Documents

The clinical sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from the participants. Medical and research records will be maintained at each clinical site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the clinical site must permit authorized representatives of the sponsor to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other participant data may be copied (and all personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to individuals. The clinical site will normally be notified before auditing visits occur.

13.5 Timing/Reports

Data will be monitored by staff at the SACCC. Status reports on the progress of the study and data collection will be generated regularly. Reports will be sent to the NIAID project manager and medical officer on a regular basis.

13.6 Study Records Retention

Study documents must be maintained at the clinical site or a local storage facility for at least 5 years following the completion of the study. Study documents that must be retained include all hard copies of CRFs, IRB approval documentation and related correspondence, and signed informed consent forms.

14 Publication Policy

Presentations and publication of the results of this study will be governed by the ICAC Publication Policy.

Appendix A: Scientific References

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Appendix B: Schedule of Procedures/Evaluations

	Quarterly Calls	11 year visit	12 year visit	13 year visit	14 year visit	15 year visit	16 year visit	Induced Sputum visit
Questionnaires ¹								
Quarterly Symptom and Exposure Assessment	X	X	X	X	X	X	X	
Annual Symptom and Exposure Updates		X	X	X	X	X	X	
Comprehensive Stress Assessment (Caretaker and Child)			X		X		X	
Study Procedures ¹								
Blood sample collection ²		X ³	X	X ³	X	X ³	X	
Physical Examination		X	X	X	X	X	X	
Urine Collection (NicAlert testing)			X		X		X	
Bioelectrical Impedance Analysis (BIA)		X		X		X		
Dust Sample Collection			X		X		X	
Fitness Test		X		X		X		
Nasal Epithelial Cell Collection		X						X
Nasal Mucus Collection					X			
IOS and Spirometry (c)		X	X	X	X	X	X	
Bronchodilator Reversibility (c)		X	X	X ⁴	X ⁵	X	X	
Methacholine Challenge (s)					X			
Induced Sputum (s)								X
Exhaled Nitric Oxide (eNO) (s)			X		X		X	
Allergen Skin Tests (e)					X			

¹ Asthma outcomes: (c) core, (s) supplemental, (e) emerging as recommended by NIAID and NHLBI Asthma Outcomes Workshop

² Additional blood may be drawn at any time during the study.

³ Blood may be collected on at the 11-, 13-, and 15-year clinic visits if not collected the previous year.

⁴ At age 13, reversibility will be tested in response to ipratropium. All other visits will use albuterol.

⁵ Bronchodilator reversibility will be performed only when the child does not meet the criteria for performing the methacholine challenge.