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

Version Number: 30.0
Protocol Addendum
Continuation of URECA Study to Age 17

21 January 2021

INVESTIGATOR SIGNATURE PAGE	
Protocol: ICAC-07 Urban Environment and Childhood Asthma	**Version/Date:** Version 30.0 / 21 January 2021
Site Principal Investigator:	
Title: Urban Environment and Childhood Asthma (URECA)	
Study Sponsor: The National Institute of Allergy and Infectious Diseases (NIAID)	
INSTRUCTIONS: The site Principal Investigator must 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). A valid electronic signature is also acceptable (e.g., sent via email as a PDF version). The original signature page must be kept for your records. Return an electronic copy of the signed signature page to the DAIT Regulatory Management Center via the applicable DAIT RMC email address for the protocol/network.	
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, 812, and in the International Conference on Harmonization (ICH) document entitled *Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance E6(R2)*. Further, I will conduct the study in keeping with local legal and regulatory requirements. 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. _____ Site Principal Investigator Printed Name _____ _____ Site Principal Investigator Signature Date	

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 E6(R2)*, 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.

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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:	N/A – 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 17 years.
Study Duration:	Start Date: 1 April 2021 End Date: 31 December 2024
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:	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:	1. To provide samples for analysis and outcomes data for use in mechanistic studies to understand relationships between environmental exposures, changes in immune development, alterations in DNA methylation patterns of airway cells, and the development of allergic sensitization, allergic rhinitis and asthma. 2. To work collaboratively with investigators outside of the NIAID asthma 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 and prevalence of asthma and occurrence of specific phenotypes of asthma

Schematic of Study Design

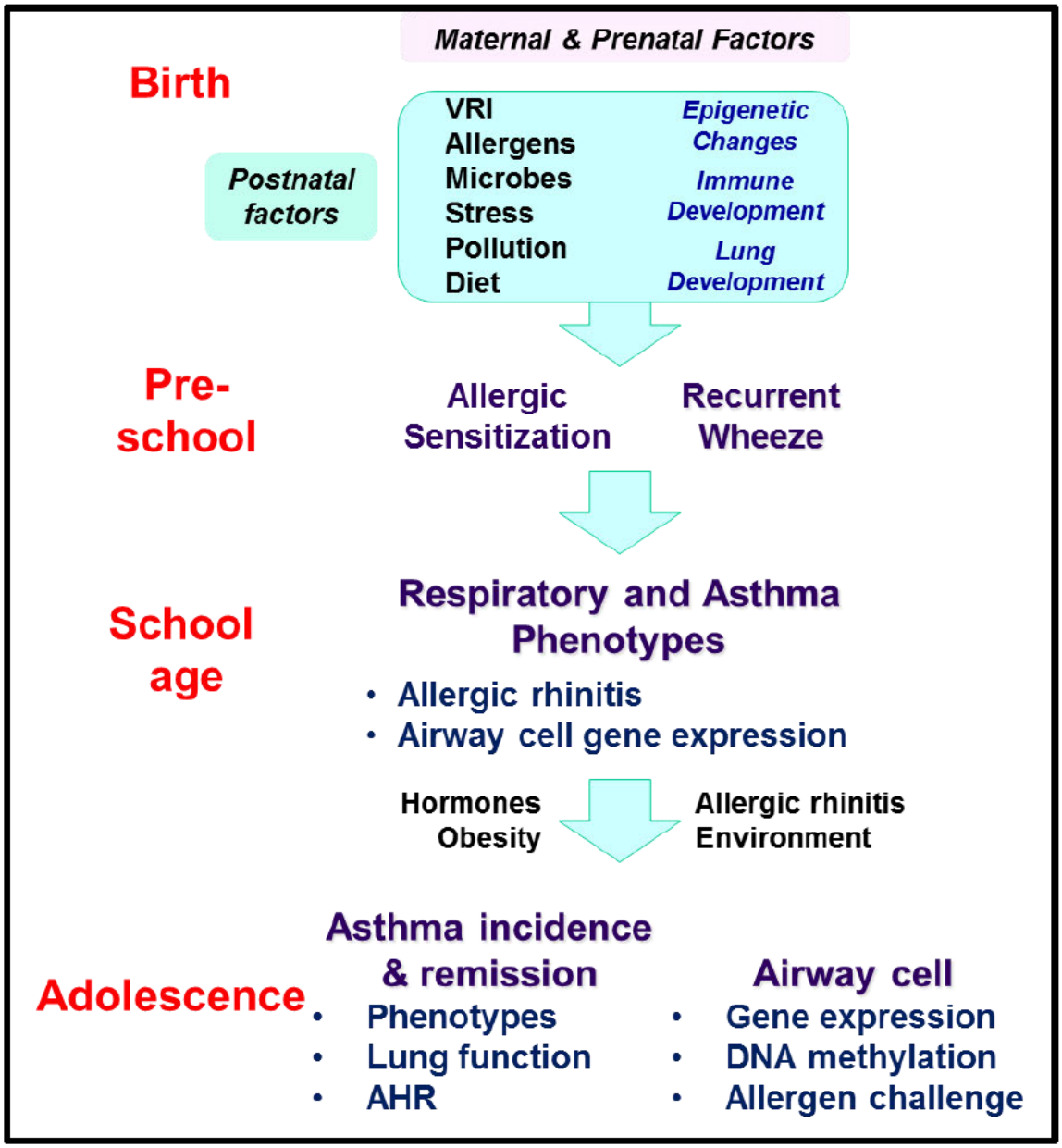


Figure 1. URECA study outcomes. The URECA study began with prenatal assessments of factors that affected early immune development and the development of allergic sensitization and recurrent wheeze during the preschool period. Analysis of data through school age is ongoing, and results to date have related early life environmental exposures to respiratory and asthma phenotypes. Analysis of early life factors that influence allergic rhinitis is in progress. The final stage of the URECA study will extend observations into adolescence, and will focus on factors related to asthma incidence and remission, further definition of allergy and asthma phenotypes, and determine relationships between early life exposures, these outcomes and mechanistic assessments of airway cell gene expression and DNA methylation.

Key Roles

PROTOCOL CHAIR – JAMES GERN, MD

[redacted]

NIAID MEDICAL MONITOR – [redacted]

[redacted]

NIAID PROJECT MANAGER – [redacted]

[redacted]

STATISTICIAN – [redacted]

[redacted]

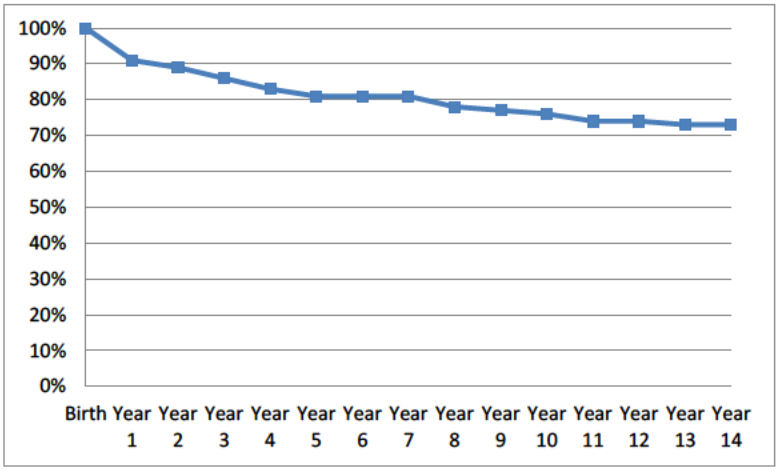
1 Background Information and Scientific Rationale

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 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] 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 youth. 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.

Retention has been highly successful. 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 **73% of the children (now ages 13-15 years) remain active participants** (Figure 1, data from November 2020).

Figure 1. URECA retention through age 14



The URECA III protocol was completed in March 2017, with the last of the 10-year clinic visits. The follow-on URECA IV protocol ends with the conclusion of ICAC funding on July 31, 2021 when the participants will be between 14 and 16 years old. The current URECA V protocol will be enacted April 1, 2021 and will allow for follow-up of the URECA participants into their 17th year.

1.2 Rationale

Minority children growing up in disadvantaged urban neighborhoods have high prevalence of asthma and burden of disease. Thus, new insights into pathologic mechanisms in early life are needed to improve and direct treatment and to enable prevention. The URECA study has

collected longitudinal information beginning at birth related to maternal asthma, key environmental exposures (allergens, pollutants, viruses, bacteria, fungi), mechanisms (immune development, airway epithelial cell gene expression, genetics) and outcomes (lung function, allergy, symptoms). Analysis of these data through age 10 years identified clinical presentations ("phenotypes") of asthma that differ by age of onset, severity, relationship to allergy, lung function and the propensity for exacerbations. For each child, the phenotype reflecting their respiratory health likely results from interactions between heritable factors and environmental exposures. **We hypothesize that personal factors (genetics, maternal asthma, hormones) interact with early life environmental exposures at airway mucosal surfaces to modify patterns of DNA methylation (DNAm) and gene expression in airway epithelial cells (AECs), and ultimately AEC function and respiratory health outcomes.** Over time, changes in epithelial cell DNAm could lead to modified airway structure and function to influence the risk of developing respiratory allergies and asthma in genetically susceptible children. Because urban minority children have unique environmental exposures and heightened asthma severity and morbidity, it is particularly important to identify specific risk factors and causal mechanisms in these children. To advance beyond recognizing the clinical expression of allergies and asthma (phenotypes) in urban children towards a comprehensive understanding of risk factors and pathologic mechanisms (endotypes), we propose the following **three specific aims** (Figure 2):

Aim 1 will test the hypothesis that genetic and environmental risks for respiratory allergy and asthma phenotypes are mediated through long-lasting epigenetic (DNAm) changes and transcriptional patterns in AECs. We will study genotypes, DNAm, and gene expression in AECs collected from URECA children at ages 11 and 15 years, together with phenotypes and exposures collected longitudinally beginning at birth.

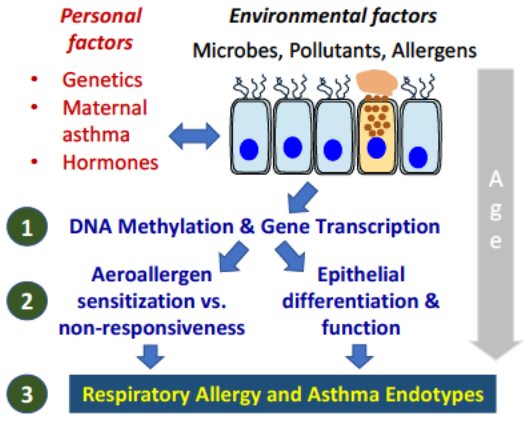


Figure 2. Specific aims (numbered).

Aim 2 will test the hypothesis that early life exposure to allergens in the home in the absence of a diverse microbiome is associated with sensitization to those allergens and respiratory symptoms (perennial rhinitis and allergic asthma phenotypes). We will identify additional early life exposures that are associated with no sensitization, sensitization without clinical symptoms and sensitization with respiratory symptoms.

Aim 3 will integrate the diverse data available in URECA children to identify asthma endotypes and their predictors by: **a)** using advanced clustering techniques to identify respiratory and asthma phenotypes from longitudinal data, including upper and lower airway symptoms, allergic sensitization, lung function, BMI and psychosocial factors such as depression and risky behaviors; **b)** linking phenotypes to potential causative factors, such as specific environmental exposures (home microbiome and allergen exposure), AEC DNAm and gene expression patterns, upper airway microbial colonization, and serum hormone levels (e.g. insulin, sex hormones, adipokines); and **c)** identifying biomarkers to enable early identification of asthma endotypes.

The results of these studies will link the pivotal early life exposures to airway epithelial cell DNAm and gene expression patterns, and ultimately to clinical expression of allergic diseases and asthma. These integrated data can be used to detect at-risk children at an early age, and from these pathways, develop and test interventions in early life to prevent allergy and asthma in urban children, and thus relieve a lifetime burden of asthma in this high-risk urban children.

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 CAUSE consortium 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

Redness, swelling, and itching of the skin are likely to occur during and 1-2 hours after an allergen skin test. 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 V Manual of Operations (MOP) for ICAC-07.

1.3.1.4 Methacholine Challenge

The 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 V 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. This procedure may cause throat irritation. Participants may rinse their mouths or gargle with water after the procedure to reduce these symptoms. Less commonly, nausea, vomiting, and headache can occur.

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 Collection of Nasal Lining Fluid

The collection of nasal lining fluid may produce mild, temporary discomfort such as a tickle nose and/or tearing of the eyes.

1.3.1.9 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 17 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 determine whether early life exposure to allergens in the home in the absence of a diverse microbiome is associated with sensitization to those allergens and respiratory symptoms (perennial rhinitis and allergic asthma phenotypes).
2. 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 determine the relationships between DNAm and gene expression in AECs collected from URECA children at ages 11 and 15 years, genotypes, and respiratory and allergy phenotypes to age 17 years.
2. To identify which urban exposures (allergens, pollutants, microbes, viral infections, stress) from early life onward are associated with AEC DNAm and gene expression (ages 11 and 15 years) and respiratory and allergy phenotypes to age 17 years.
3. To work collaboratively with investigators in CREW, ECHO and other networks 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 to age 17 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 14 and 17 years, and thus, this stage of URECA will collect data on not only the development and resolution of asthma but also asthma morbidity and changes in 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.

3.2 Study Endpoint

3.2.1 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. At age 7, 29% of the URECA study participants met this definition for asthma.[4] We also identified five specific phenotypes based on longitudinal trajectories of symptoms, allergen sensitization, and lung function using data through age 7 years.[5] These trajectories were refined at age 10 years, where an additional phenotype with high symptoms, high sensitization, and significant lung function deficits was found (Altman M et al, under review).

Data regarding asthma diagnosis, the frequency and severity of cough and wheezing illnesses, healthcare utilization, and medication use will continue to be collected during each of the quarterly telephone calls. Examinations will also be conducted as part of the scheduled study visits to age 17 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.

3.2.2 Secondary Endpoints

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

1. Methacholine responsiveness – this will be ascertained at the 14/15-year time point.
2. Pulmonary function – pre- and post-bronchodilator spirometry and impulse oscillometry will be measured at age 14/15 and again at age 16/17.
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

4.1 Population Description

To address the primary objectives of the URECA V protocol, all the participants active in the URECA IV protocol in early 2021 will be invited to continue under the new protocol into age 17 years.

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 V are those who are active in the URECA IV protocol at approximately age 14-16.

5 Study Procedures/Evaluations

5.1 Clinical Evaluations

A number of procedures are currently on hold due to the COVID pandemic, and we will maintain flexibility in scheduling based on when research restrictions related to the pandemic are lifted. For example, if any of the scheduled assessments do not occur at a visit, that assessment can be completed at the next visit after restrictions are lifted. 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 the age 16/17-year time point following procedures described in the URECA V MOP for ICAC-07. One combined dust sample will be collected from the participant's bed (defined as the location where the participant 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 the 14/15-year time point. This 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 V MOP for ICAC-07 will perform this test. Skin testing will be done by the prick technique using the Greer*Pick* system (Greer; Lenoir, NC) in accordance with generally accepted guidelines.[6] 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 V MOP prior to the test to limit interference with the results of the skin test.

5.1.3 Pre/Post Bronchodilator Pulmonary Function Testing

The 14/15- and 16/17-year time points will each include one pre/post bronchodilator testing session. 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.

5.1.4 Methacholine Challenge

Participants will undergo methacholine challenge testing one time. Airway responsiveness will be measured by assessing the dose of methacholine required to produce a drop in FEV_1 of 20% (PD_{20}) after the administration of increasing concentrations of methacholine using the small volume nebulizer-tidal breathing technique. A trained and certified pulmonary function technician will perform the test based on the procedures outlined in the URECA V MOP for ICAC-07. These procedures will be used 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 two visits, once in the 14/15-year time point and once in the 16/17-year time point. The procedure will be conducted prior to the measurement of spirometry. Exhaled NO will be measured employing a technique modified after Silkoff et al.[7] and following American Thoracic Society (ATS) guidelines for eNO assessment.[8] Nitric oxide concentrations will be measured using a commercially-available analyzer and a procedure described in detail in the URECA V MOP for ICAC-07.

5.1.6 Induced Sputum

Sputum will be induced at the age 16/17-year time point by inhalation of hypertonic saline solution using the method that was used in the Asthma Clinical Research Network (ACRN).[9] Safety monitoring per spirometry and symptom report will be performed during and after sputum induction. Processing will be performed according to the URECA V 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 brushing the nasal mucosa at the age 14/15-year time point. 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 V MOP for ICAC-07.

5.1.8 Collection of Nasal Lining Fluid

Nasal lining fluid will be collected at the age 14/15-year time point, by placing absorbent filter paper just inside the nasal cavity, allowing for passive collection of secretions. This sample will be obtained for future measurements of metabolites, proteins, and possibly microbiome.

5.1.9 Fitness Testing

The Six-Minute Walk Test will be used at the 14/15-year time point to assess the participant's level of fitness.[10] For this test the participant 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 participant's pulse and respiratory rate. The participant 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 will not result in withdrawal from the study, but will be reported on the appropriate CRFs and followed to outcome. Pregnant participants may not participate in some procedures; refer to the URECA V MOP for ICAC-07. 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 14/15, and 16/17-year time points for measurement of urinary cotinine, a marker for tobacco smoke exposure. A urine sample will be collected following procedures described in the URECA V MOP for ICAC-07.

5.2.1.3 Blood Collection

Blood will be collected while participants are active in URECA V 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. In addition, systems approaches (e.g. microarray, proteomics, epigenetics) will also be used, 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) or metabolites 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 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 participant nor their caregivers can be identified individually.

5.2.2.2 Specimen Shipment

Instructions for sample preparation, handling, storage, and shipping are included in the URECA V 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 research groups identified by the CAUSE Leadership Center, 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 the University of Chicago), and
3. Analysis of epitope recognition and functional characteristics of allergen-specific T cells, and relationship to allergen exposure, sensitization, and expression of clinical disease (collaboration with the La Jolla 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. 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 CAUSE Steering Committee and DAIT/NIH to destroy them.

7 Study Schedule

The COVID pandemic has caused unpredictable delays in the visit schedule. To maximize participation and facilitate the collection of complete data sets in URECA V, we have designed the protocol with increased flexibility in scheduling. This will include two visits in the 14/15-year time frame, two visits in the 16/17-year time frame, and one standalone visit for a subset of participants. Many procedures that cannot be completed – due to COVID-related restrictions, for example – at their intended visits may be completed at the subsequent visit (see Appendix B).

7.1 Informed Consent

Once the URECA V protocol is approved, informed consent to continue participation will be obtained by the URECA staff at the earliest time that the participant's parent/guardian is seen in person. If the family has not been seen in person and the participant reaches 16 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 at the next clinic visit, before any study activity. We will also obtain written assent from participating children according to local IRB guidelines before enrollment into URECA V.

7.2 14/15-Year Clinic Visit A

At the 14/15-year Visit A, questionnaires about the participant's physical health, habits, and environment will be administered. Questionnaires pertaining to stress and mental health will also be administered. A brief physical examination will be conducted, to focus on ears, nose, throat, chest and skin, and including Tanner staging. Urine and blood samples will be collected, supplies and instructions for home dust collection will be distributed, and exhaled nitric oxide will be measured. IOS and spirometry will be performed. Allergen skin testing to a panel of common indoor and outdoor allergens will be performed. Methacholine challenge is planned, with post-bronchodilator spirometry and IOS reversibility being done only when participants do not meet the criteria for methacholine challenge.

7.3 14/15-Year Clinic Visit B

At the 14/15-year Visit B, questionnaires about the participant's physical health, habits, and environment will be administered. A brief physical examination will be conducted, to focus on ears, nose, throat, chest and skin. Urine samples will be collected for post-menarcheal female participants. BIA will be performed to assess adiposity. In addition, the Six-Minute Walk Test will be performed to assess fitness. Nasal lining fluid will be collected in addition to nasal epithelial cells. IOS and spirometry will be performed. Participants who were unable to conduct the methacholine challenge at Visit A will attempt to conduct the procedure at this visit, again with post-bronchodilator spirometry and IOS reversibility done if the participant does not meet the criteria for the challenge. If the methacholine challenge is not conducted at either Visit A or Visit B during this time point, it will be conducted at a separate visit to occur any time after the 14/15-year Visit B.

7.4 16/17-Year Clinic Visit A

At the 16/17-year Visit A, questionnaires about the participant's physical health, habits, and environment will be administered. Questionnaires pertaining to stress and mental health will also be administered. A brief physical examination will be conducted, to focus on ears, nose, throat, chest and skin. Urine and blood samples will be collected. Spirometry and sputum induction will be performed. Supplies and instructions for the home dust collection will be provided to the families.

7.5 16/17-Year Clinic Visit B

At the 16/17-year Visit B, questionnaires about the participant's physical health, habits, and environment will be administered. A brief physical examination will be conducted, to focus on

ears, nose, throat, chest and skin. Urine samples will be collected for post-menarcheal female participants. BIA will be performed to assess adiposity. Spirometry and impulse oscillation spirometry with reversibility will be performed. Exhaled nitric oxide will be measured.

7.6 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. If the final 16/17-year visit (Visit B) has occurred, but the child has not yet reached the age of 17 years, quarterly calls may continue until the 17th year. 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.

7.7 Methacholine Challenge Visit

Participants who are unable to complete the methacholine challenge at their 14/15-year Visit A or Visit B will be asked to come to clinic for a separate visit. During this visit, questionnaires will be administered to assess for changes to the participant's health and medications. The full set of questionnaires for annual clinic visits is not expected, although some may be administered if previously missed. IOS and Spirometry will be performed, followed by methacholine challenge. If the participant does not meet criteria to perform the challenge, post-bronchodilator spirometry and IOS with reversibility may be completed, if not previously conducted at the 14/15-year time point.

7.8 Home Environmental Exposure Evaluation

At the 16/17-year Visit A, the family will be given a dust collection kit, which includes instructions on how to collect a dust sample (refer to the URECA V Dust Collection Instructions Handout). A combined dust sample from the participant's bedroom floor and the participant's bed will be collected. The room where the participant 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 or participant 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.

7.9 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. 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.

7.10 Re-contact of Subjects after Study Termination

The caretaker/guardian of the 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 two 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 Data Safety and Monitoring Board (DSMB). 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 continuation into age 17 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 age 14/15.
2. Pulmonary function – pre- and post- bronchodilator spirometry and impulse oscillometry will be measured once at age 14/15, and once at age 16/17.
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 73% of the original cohort still active at age 14/15. In recent phases of URECA, >85% 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).[11-13] 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.[12-18] 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.[19] 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 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 17 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. Using longitudinal data obtained from the URECA cohort through the age of 17 years, we will perform a cluster analysis to identify patterns of wheezing, allergic sensitization and lung function over time. In URECA the comprehensive longitudinal measurements within a child allow the application of techniques such as 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. We will consider latent class analysis and other clustering techniques to identify phenotypes in URECA V.

10.4.2 Analysis of Secondary Objectives

10.4.2.1 Early life exposure to household allergens

To determine whether early life exposure to allergens in the home in the absence of a diverse microbiome is associated with sensitization to those allergens and respiratory symptoms (perennial rhinitis and allergic asthma phenotypes), we will examine the association between early-life exposures to allergens and diversity in the microbiome towards allergic sensitization. The assumption is that children with high levels of exposure and low microbiome diversity will have a higher likelihood of early sensitizations. Early sensitizations will be obtained by latent class mixed model analysis combining both skin tests and specific IgEs to aeroallergens. Then a multinomial logit model, with possible adjustments for race and gender, will test the (multiplicative & additive) interaction between these two predictors and the likelihood of early sensitization. Furthermore, these prolonged exposures can have a compound effect in sensitization, and summary measures such as cumulative exposures may not fully capture these relationships. We proposed to extend the model suggested above by using a distributed lag linear (and non-linear) model with flexible exposure-response and lag-response functions to examine the association between sensitization and exposures in early life.

Pathway analysis will be used to determine how a host of environmental factors contribute to the development of rhinitis with specific classifications. Based on medical evidence in the

published literature we will develop and test a conceptual model to describe how different risk factors domains (i.e. allergic inflammation, pulmonary physiology, environmental exposures, etc.) are linked to rhinitis. A casual network analysis will be implemented using structural equations models to simultaneously perform (1) a confirmatory factor analysis in each domain to estimate the relationship between the domain of interest and each observed variable used to measure the domain and (2) a series of regression models to estimate the standardized direct effect of each independent domain onto rhinitis.

10.4.2.2 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, 14/15, and 16/17 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 from age 10 to 17 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.

10.4.3 Analysis of Exploratory Endpoints

The overall approach to identify phenotypes will be similar to that used with the 7-year and 10-year data sets, using latent class mixed models to identify homogenous subpopulations within sets of longitudinal variable trajectories. Variables characterizing the trajectory clusters (e.g. symptoms, allergic sensitization, lung function and BMI) and other relevant predictors (including demographics) will be used to represent subjects in a Gower distance matrix, which can handle mixed data types. The Akaike information criterion will be used to select the number of clusters (i.e. number of subpopulations) for each variable. These variables will be evaluated for collinearity and variable sets that are highly collinear will be represented by a constituent variable that is most representative of each subset. Children will be clustered to identify distinct phenotypes selected using four criteria: 1) a generalization of the within-clusters sum of squares measure, 2) average silhouette width, 3) Dunn index, and 4) the ratio of average-within versus average-between clusters. In this aim, we will identify asthma endotypes by linking respiratory allergy and asthma phenotypes to mechanistic data, including the patterns of airway epithelial cell DNA methylation and gene expression at 11 and 15 years that will be calculated in Aim 1. Our approach will first assign each subject to one of the phenotypes defined by the approach for Subaim 3.1. We will then conduct an analysis to identify gene expression patterns and

methylation patterns (from ages 11 and 15), and environmental exposures that are characteristic of each phenotype and distinguish among them.

To identify endotypes, we will use a sparse multinomial logistic regression model Variables for these models include the DNA methylation and expression modules (Aim 1), and environmental exposures (e.g. viral infections, allergens, microbes, tobacco smoke). Thus, the model will represent endotypes by mapping from biological states and environmental exposures to clinical phenotypes. In a sparse model, the learning algorithm is biased towards selecting a relatively small number of variables. Thus, although we will include a moderately large number of candidate variables in the models, only a sufficient number of variables to distinguish the phenotypes are selected. We will use well established sparse learning methods, such as LASSO and elastic net, which are well suited to high-dimensional data sets that include many potentially irrelevant variables.

We will next identify biomarkers for predicting respiratory allergy and asthma phenotypes that we observe at ages 11 and 15. This analysis will start with subjects clustered according to the phenotypes that we defined in Aim 3.1 and will proceed by inferring functions composed from individual early-life biomarkers that are predictive of the phenotype an individual subject will exhibit. Sparse multinomial logistic regression models will be used to evaluate early life biomarkers. The candidate predictor variables for these models will include (i) demographics, (ii) clinical variables such as wheeze frequency, (iii) gene expression measurements from nasal epithelial cells, and (iv) DNAm measurements from nasal epithelial cells. Aside from demographics, these variables will come from measurements taken during the first three years of life.

10.5 Sample Size Considerations

The sample size for the URECA cohort is determined by the original recruitment, and retention to this point (442 subjects at age 14 years, which represents 73% of the original 609 recruited subjects) has been excellent. This sample size enabled identification of phenotypes at 7 and 10 years of age.

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 V 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 they are responsible for conducting. Details of the quality control plan, including certifications and quality control of study procedures, are provided in the URECA V 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 V participants 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 V 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 CAUSE Publication Policy.

Appendix A: Scientific References

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Appendix B: Schedule of Procedures/Evaluations

In response to the scheduling challenges posed by COVID-19, the URECA V protocol is written so that many visit activities can shift from one visit to the next. If a participant has consented to additional visits, activities may also be made-up at unscheduled, additional visits.

- ● Formally scheduled at this visit
- ◁ May be conducted at this visit only if missed when last formally scheduled.

	Quarterly Calls	14/15-Year Time Point		16/17-Year Time Point		Methacholine Challenge Visit [2]
		Visit A	Visit B	Visit A	Visit B	
Questionnaires[1]						
Quarterly Symptom and Exposure Assessment	●	●	●	●	●	
Annual Symptom and Exposure Updates		●	●	●	●	
Comprehensive Stress Assessment *(Caretaker and Child)*		●	◁	●	◁	◁
Study Procedures[1, 3]						
Allergen Skin Test (e)		●	◁	◁	◁	◁
Bioelectrical Impedance Analysis			●		●	◁
Blood Sample Collection[4]		●	◁	●	◁	◁
Dust Sample Collection Supply Distribution		●	◁	●	◁	◁
Exhaled Nitric Oxide (s)		●	◁		●	◁
Fitness Test			●			
Impulse Oscillometry		●	●		●	●
Induced Sputum (s)				●		
Methacholine Challenge (s)		●	◁			●
Nasal Epithelial Cell Collection			●	◁	◁	◁
Nasal Lining Fluid Collection			●	◁	◁	◁
NicoTest *(Urine collection)*		●	◁	●	◁	◁
Physical Examination		●	◁	●	◁	●
Post-Bronchodilator IOS and Spirometry (c)		●[5]	◁[5]		●	◁[6]
Pregnancy Test *(Urine collection, as applicable)*		●	●	●	●	●
Spirometry (c)		●	●	●	●	●
Tanner Stage		●	◁			◁

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

[2] As applicable. Participants who are unable to conduct the methacholine challenge at the 14/15-Year Visit A or Visit B will be asked to come to clinic for a separate visit any time after the participant's 14/15-Year Visit B.

[3] Invalid tests or samples that are lost, damaged, or otherwise unusable may be repeated at either an unscheduled visit or the following clinic visit.

[4] Additional blood may be drawn at any time during the study.

[5] At the Year 14 visit, bronchodilator reversibility will be performed only if the child does not meet the criteria for performing the methacholine challenge. In this case, the methacholine challenge will be conducted at Year 15. If,

instead, bronchodilator reversibility is missed at Year 14 in favor of the methacholine challenge, reversibility will be done at the Year 15 visit.

[6] At the standalone Methacholine Challenge Visit, staff will conduct Post-Bronchodilator IOS/Spirometry only when the participant both does not meet ATS guidelines for the Methacholine Challenge and did not perform Post-Bronchodilator IOS/Spirometry at the last formally scheduled instance.