

TECHNICAL REPORT

The Tennessee Children's Respiratory Initiative: Objectives, design and recruitment results of a prospective cohort study investigating infant viral respiratory illness and the development of asthma and allergic diseases

TINA V. HARTERT,^{1,2,3,4} KECIA CARROLL,^{3,5} TEBEB GEBRETSADIK,^{3,6} KIMBERLY WOODWARD,^{1,3} PATRICIA MINTON^{1,3} AND THE VANDERBILT CENTER FOR ASTHMA AND ENVIRONMENTAL HEALTH RESEARCH INVESTIGATORS AND COLLABORATORS*

Departments of ¹Medicine, ⁵Pediatrics, ⁶Biostatistics, and ²the Institute for Medicine and Public Health, ³Center for Asthma Research and Environmental Health, ⁴General Clinical Research Center, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

ABSTRACT

Background and objective: The 'attack rate' of asthma following viral lower respiratory tract infections (LRTI) is about 3–4 fold higher than that of the general population; however, the majority of children who develop viral LRTI during infancy do not develop asthma, and asthma incidence has been observed to continuously decrease with age. Thus, we do not understand how viral LRTI either predispose or serve as a marker of children to develop asthma. The Tennessee Children's Respiratory Initiative has been established as a longitudinal prospective investigation of infants and their biological mothers. The primary goals are to investigate both the acute and the long-term health consequences of varying severity and aetiology of clinically significant viral respiratory tract infections on early childhood outcomes.

Methods: Over four respiratory viral seasons, 2004–2008, term, non-low birth weight previously healthy infants and their biological mothers were enrolled during an infant's acute viral respiratory illness. Longitudinal follow up to age 6 years is ongoing.

Results: This report describes the study objectives, design and recruitment results of the over 650 families enrolled in this longitudinal investigation. The Tennessee Children's Respiratory Initiative is additionally unique because it is designed in parallel with a large retrospective birth cohort of over 95 000 mother–infant dyads with similar objectives to investigate the role of respiratory viral infection severity and aetiology in the development of asthma.

SUMMARY AT A GLANCE

This report describes the study objectives, design and recruitment results of the Tennessee Children's Respiratory Initiative, designed to investigate the role of respiratory viral infection severity and etiology in asthma development. Future reports will address the complex relationship between infant respiratory viral infection and asthma and atopic diseases.

Conclusions: Future reports from this cohort will help to clarify the complex relationship between infant respiratory viral infection severity, aetiology, atopic predisposition and the subsequent development of early childhood asthma and atopic diseases.

Key words: allergic rhinitis, asthma, bronchiolitis, Immunoglobulin E, respiratory virus.

INTRODUCTION

The Tennessee Children's Respiratory Initiative (TCRI) has been established as a longitudinal prospective investigation of term, non-low birth weight otherwise healthy infants and their biological mothers. The primary goals of the study are: (i) to investigate both the acute and the long-term health consequences of varying severity and aetiology of clinically significant viral respiratory tract infections on the outcomes of allergic rhinitis (AR) and early childhood asthma; and (ii) to identify the potentially modifiable factors that define children who are at greatest risk of developing asthma following infant respiratory viral infection. This study is unique, in that it was designed in parallel with our Tennessee Asthma Bronchiolitis Study (TABS), which is a retrospective birth cohort study of over 95 000 infants and their biological mothers similarly designed to elucidate the factors predisposing to childhood asthma and allergic

Correspondence: Tina V. Hartert, Vanderbilt University School of Medicine, 6107 Medical Center East, Nashville, TN 37232-8300, USA. Email: tina.hartert@vanderbilt.edu

*The collaborators of this study are listed in the acknowledgments section.

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diseases, but lacking biospecimens. Thus, we designed the prospective TCRI to establish a base for the evaluation of both the risks and benefits of documented significant infant viral respiratory infection of varying severity and aetiology and other environmental exposures on childhood atopy outcomes and to establish a biospecimen repository for analyses including biomarker testing and genotyping. The prospective cohort has the longitudinal design properties that may overcome potential limitations intrinsic to retrospective studies, such as our TABS cohort.^{1–5} It is our eventual goal that the findings from these investigations, in conjunction with other investigations that have helped to elucidate genetic and environmental factors associated with asthma development, will help in the identification of primary and secondary prevention strategies for asthma. This report describes the study objectives, design and recruitment results of this study cohort.

METHODS

Study objectives and design

The TCRI is a prospective cohort of mother–infant dyads enrolled in a longitudinal investigation of the

relationship of infant viral respiratory infection severity and aetiology and the interaction of other risk factors on the development of childhood asthma and allergic diseases. The study was approved by the Vanderbilt Institutional Review Board, and parents provided written informed consent for both their and their child's study participation.

Subject recruitment and study population

Term, non-low birth weight, otherwise healthy infants were enrolled along with their biological mothers, at a single academic institution, Vanderbilt Children's Hospital, at the time of an acute visit (hospitalization, emergency department or unscheduled outpatient visit) for presumed viral bronchiolitis or upper respiratory tract infection (URI) during respiratory viral seasons September through May 2004–2008. Inclusion and exclusion criteria are outlined in Table 1. Because of the grant funding start date, the first study season did not begin until November 2004. Recruitment was solely hospital- and clinic-based, and was performed 7 days/week during the first 2 years of cohort accrual, and 5 days per week for the two subsequent years. Screening and recruitment were done by experienced

Table 1 Tennessee Children's Respiratory Initiative enrolment inclusion and exclusion criteria

Enrolment inclusion criteria	Operational definition
Gestational age	≥37 weeks
Birth weight	≥2275 grams
Previously healthy infant	See exclusions
Birth—12 months of age	Birth through 12 months of age during the acute respiratory illness enrolment visit
Biological mother available	Biological mother available to complete skin testing and questionnaire
Presumed viral bronchiolitis, LRTI, or URI	Based on both admitting physician diagnosis AND documentation of symptoms with duration <11 days (including any two of the following: cough, nasal congestion, rhinorrhea, wheezing, dyspnea, fever) and requirement for either hospitalization, including 23-h stay, ED visit or acute care clinic visit
Enrolment exclusion criteria	
Adopted or foster child	
Unable to determine maternal history	
Significant comorbidities or cardiopulmonary disease	Congenital or acquired chronic heart or lung disease, prior requirement for mechanical ventilation for cardiac or pulmonary disease, immunodeficiency, neurologic disease with possible aspiration, significant gastroesophageal reflux disease felt to contribute to pulmonary disease, tracheomalacia
Ever received one or more doses of RSV-IVIG or palivizumab	
Prior study inclusion	
Fever and neutropenia	
Children whose parents or guardians were not able to understand the consent process, or a language barrier [†]	

[†] Spanish speaking families were enrolled during the first 2 years of the study, and not thereafter because of lack of trained bilingual research personnel.

LRTI, lower respiratory tract infections; URI, upper respiratory tract infection.

research nurses using computerized medical charts to screen infants with presumed respiratory viral illness.

Examination components of the enrolment visit

The components and time line of the subject visits are outlined in Table 2. The trained research nurses administered a structured questionnaire during an in-person interview. The questionnaire was used to obtain information on demographics, acute medical symptoms, previous medical history, infant feeding method, development, comorbid conditions, detailed family history of asthma and atopic diseases using a family tree form routinely used in genetic epidemiology studies, responses to the International Study of Asthma and Allergies in Children (ISAAC) questionnaire, home exposures including pets and detailed smoking exposure, ill contacts, number of siblings and day-care attendance.⁶⁻⁸ A pilot sample of mothers of hospitalized infants also completed the Block 2000-Brief food frequency questionnaire, Nutritionquest, Berkeley, CA.⁹⁻¹¹ Infant biospecimen collection included nasal and throat swabs for viral detection, and bag urine samples for biomarker determination. Mothers underwent prick skin testing to eight common aeroallergens and a blood specimen was obtained by venepuncture for serum immunoglobulin E (IgE) and DNA. A structured abstraction form was used to obtain information from the medical record regarding the index enrolment visit: current infant weight, confirmation of birth weight, room air pulse oximetry, requirement for supplemental oxygen, medication administration, prior wheezing episodes and detailed information on the current illness and hospital course. Following discharge from the hospital or outpatient setting, the final discharge diagnosis and results of culture data were obtained through chart review.

Cohort follow up

There are three phases of cohort follow up. First, mothers and children undergo an in-person well-child follow-up visit during the child's second year of life conducted in the Vanderbilt Clinical Research Center, or during a home visit. Second, families are re-contacted every 12–18 months by phone and/or mailings for purposes of cohort retention, and to provide reminders about the remaining study components. Third, mothers, or the current guardians, undergo a phone interview during the fourth and sixth years of life to identify children with asthma, transient wheezing, AR and eczema.

Examination components of the second year well-child follow-up visit

The 2-year in-person well-child visit is conducted in the Vanderbilt Clinical Research Center, or during a

home visit offered to those unable to return to the study institution. During the visit, the ISAAC questionnaire is administered, blood samples are obtained from children and mothers (if not previously collected) and a buccal swab is collected for DNA if blood cannot be obtained.

Examination components of the fourth and sixth year of life questionnaire

A structured telephone questionnaire is administered to the mother/parent when their child is 4 and again at 6 years. Trained interviewers employed in the Vanderbilt Survey Research Shared Resource use a web-based computer system to conduct structured telephone interviews, which capture detailed information on asthma and atopy diagnoses and symptoms, extensive environmental exposure history, physical activity, and comorbidities. Asthma, AR and atopic dermatitis outcomes are determined using the ISAAC questionnaire. For children with report of asthma and/or asthma symptoms in the previous 12 months, the Asthma Therapy Assessment Questionnaire is administered, and information on asthma medications, and asthma-related health-care visits are sought.^{12,13}

Biospecimen collection and laboratory analyses

Table 3 outlines the details of cohort biospecimen collection, repository and testing, which includes infant nasopharyngeal, urine, blood and nasal epithelial cell sample collection, and maternal prick skin testing and blood samples.

Ascertainment of acute respiratory illness, childhood asthma and allergic diseases

Acute respiratory illness type and severity

The discharge diagnosis and supporting clinical parameters of the infant acute respiratory illness visit were reviewed to confirm whether each child had lower respiratory tract infections (LRTI) or URI ($n = 628$) or another diagnosis ($n = 46$). LRTI and URI were defined using both the physician discharge diagnosis, as well as post-discharge chart review, and those cases that were not clearly identified as either LRTI or URI were reviewed by a panel of paediatricians who determined whether the illness represented an LRTI, URI, croup or other, which included those that could not be categorized with the available clinical information.

Acute respiratory illness severity was determined using the ordinal bronchiolitis score that incorporates admission information on respiratory rate, flaring or retractions, room air oxygen saturation and wheezing, into a score ranging from 0 to 12 (12 being most severe).^{27,28}

Table 2 Components of the Tennessee Children's Respiratory Initiative enrolment visit, and each follow-up contact

	Enrolment	1–2-year-old well-child follow-up visit			
		Year 1–year 3	Year 4–year 5	Year 6 follow up	
Time line	0–12-month-old term, non-low birth weight otherwise healthy infant enrolled at the time of a respiratory viral illness [†]	2-year-old well-child visit conducted in the CRC or during home visit	Phone contact	Phone contact	Phone contact
Maternal skin testing [†]	Sept–May 2004–2008	2005–2009	2005–2011	2008–2012	2010–2014
Questionnaire and chart review	✓			✓	✓
Administration of structured phone questionnaire	✓				
Infant nasal and throat swab for viral identification	✓				
Infant urine specimen	✓				
Family history, including detailed atopic history	✓				
Infant nasal epithelium (for cell culture repository)	✓				
Child serum IgE and allergen specific IgE determination	✓ (or)				
Infant DNA	✓ (or)				
Maternal DNA	✓			✓	
Routine phone/mailling contact		Every 12–18 months		✓	✓

[†] These infant–mother dyads were recruited and enrolled at Vanderbilt Children's Hospital (VCH), VCH paediatric emergency department, and VCH paediatric acute care clinic.

[‡] Allergen specific IgE (Phadiatop) is performed if unable to perform prick skin testing or negative histamine/positive control. CRC, Clinical Research Center; IgE, immunoglobulin E.

Table 3 Tennessee Children's Respiratory Initiative biospecimen collection and laboratory analyses

Biospecimen type	Collection timepoint	Maternal or child biospecimen	Collection and analyses
Nasopharyngeal swab for identification of viral pathogens	Enrolment	Infant	Two nasal and throat swabs are obtained from infants at enrolment during the acute illness using Dacron swabs placed in both Hank's viral transport media and lysis buffer. The biospecimens are processed, aliquoted and stored at -80°C for en batch testing for RSV A and B, human RV, adenovirus, hMPV, coronaviruses, influenza A and B, and parainfluenza types 1, 2 and 3, using the described molecular techniques. First nucleic acid extraction is performed with a Roche MagNAPure LC automated instrument that is capable of high-throughput specimen processing. The laboratory has developed highly sensitive and specific qRT-PCR assays for many common respiratory viruses, including hMPV, HCoV, RSV, influenza A and B, parainfluenzaviruses 1-3 and rhinovirus. Real-time RT-PCR is performed using the Cepheid Smart Cycler II. All specimens are first tested for GAPDH to confirm integrity of RNA and monitor for potential PCR inhibitors. Negative and positive controls are included with each run, including RNA runoff transcripts to generate a quantitation curve. ¹⁴⁻²¹ If rapid antigen and/or culture for RSV or influenza were performed at the discretion of the admitting physicians during the index visit, these results are also captured and entered in the database.
Maternal prick skin testing or specific IgE determination	Enrolment	Maternal	Maternal prick skin testing was performed by trained research nurses. Women were first screened for pregnancy, and those who were not pregnant or who had no other contraindication to skin testing underwent prick skin testing to saline, histamine and eight aeroallergens: cat pelt, Alternaria, Grass Mix #7, Ragweed mix, Oak mix, Tricophyton, Mite mix, cockroach mix (Quintest Extract Tray, HollisterStier, Spokane, WA). Phadiatop was also performed on maternal blood samples for women who could not undergo prick skin testing, or who had an inadequate skin test.
Specific and total IgE	Follow-up well-child visit	Infant	The total serum IgE and multi-allergen screen (Phadiatop, Phadia, Kalamazoo, MI) were measured on the ImmunoCAP250. Data were reported in kIU/L (total IgE) where 1 IU = 2.42 ng and kUa/L for the multi-allergen screen. The Johns Hopkins DACI laboratory will perform these tests and is a Federally licensed (CLIA-88 certified) laboratory. ^{22,23} A positive Phadiatop is defined as ≥ 0.35 kUa/L.
Nasal epithelial cells	Follow-up well-child visit	Child	Primary cultures of nasal respiratory epithelia are established by methods developed for the cultivation of epidermal keratinocytes with modifications and collected to examine innate immune response and phenotypic differences to <i>in vitro</i> infection among cells from infants with LRTI v URI. ²⁴ The cells are collected using Mid Turbinate Peds Nylon Flocked Swabs (MicroRheologics, Brescia, Italy), and placed into collection and digestion media, followed by processing and plating onto collagen IV coated tissue culture and grown at 37°C in a 5% CO ₂ incubator. To develop techniques for isolation, short-term culture, and <i>in vivo</i> modelling of epithelial and stromal cells, a xenograph model has been developed. Following short-term growth in culture, cells are transferred to denuded rat tracheas and implanted subcutaneously in nude mice.
DNA	Follow-up well-child visit	Mother and child	DNA is collected from both the mother and child during the blood collection, or using a buccal swab if blood can not be obtained. DNA is extracted by the Vanderbilt Center for Human Genetics Research Core Laboratory and stored following extraction for future studies.
Urine	Enrolment	Infant	Urine is collected from hospitalized infants during the acute infant illness at study enrolment, and from a convenience sample of outpatient subjects. Urinary measurements including, leukotrienes C ₄ /D ₄ /E ₄ (LTC ₄ /D ₄ /E ₄), and urinary metabolite of the isoprostan, 15-F _{2t} -IsoP (8-iso-PGF _{2α}) will be measured by a gas chromatographic, and other biomarkers and the remainder of the urinary biospecimens will be maintained in the repository. ^{25,26}

IgE, immunoglobulin E; LRTI, lower respiratory tract infections; URI, upper respiratory tract infection.

Familial, maternal and child atopic status

The family history of atopy was obtained using a family tree. Maternal atopy will be categorized as evidence of atopy by skin testing or specific IgE, and/or clinical symptoms of an atopic disease as assessed by the ISAAC questionnaire. Atopic status of the child will be determined by laboratory evidence of specific IgE during the second year of life, and by clinical evidence based on the above definitions.

Childhood asthma

The diagnosis of asthma will be determined at age 6 years based on responses to the ISAAC questionnaire.^{6–8} The following criteria will define asthma during the sixth year of life: (i) 12-month prevalence of symptoms of asthma (current wheeze) or the presence of exercise-induced wheeze or dry cough at night not due to a cold or chest infection; and (ii) physician diagnosis as determined by the ISAAC questionnaire using either parental reported physician diagnosis of asthma or chronic use/prescription of asthma-specific medications. Probable asthma will be defined as physician diagnosis only and analysed separately.

Transient early wheezing will be defined as wheezing episodes present in the first 4 years of life, but not meeting the definition for childhood asthma at age 4 and 6 years.²⁹

Allergic rhinitis will be determined through the ISAAC core questions on AR.^{7,8} Children will be considered to have definite AR if each of three conditions is present between age 5 and 6 years: (i) a history of nasal congestion, runny nose, itchy watery eyes, sinus pain or pressure or headaches, sneezing, blocked nose, loss of sense of smell; (ii) substantial variability in symptoms over time or seasonality; and (iii) diagnosed as having allergic rhinitis by a physician or on medications for AR. Probable allergic rhinitis will be defined as meeting two of the three criteria, or only criteria 3.

Atopic dermatitis will be determined through the ISAAC core questions on atopic dermatitis, which are based on a list of major and minor criteria widely applied in clinical studies.^{8,30,31} As eczema is probably more readily confirmed by objective tests than either asthma or rhinitis, patients will be considered to have definite atopic dermatitis if between age 5 and 6 years they report ever having an itchy rash that comes and goes for at least 6 months, and being diagnosed with eczema by a physician.^{30,31} Probable atopic dermatitis will be defined as one of the two above criteria.

Quality-control procedures

In order to standardize and monitor the quality of data collection and processing, all study personnel received training and were certified for all the study procedures. Information is recorded on paper case report forms, data are entered and then checked by a

second reviewer. Logical data checks are programmed and additionally performed by our systems analyst, investigators and again by our biostatisticians. For laboratory analyses, blind quality-control samples are included in each biospecimen run. Telephone interviewers complete classroom training, orientation to the study population, computer modules, role play interviewing and training on study-specific protocols, and are formally evaluated at the end of training. A verbatim-recording of the interviewer and participant responses, and 10% participant re-contact allows quality-control staff to verify responses.

Statistical analyses

The outcome variables of interest are the incidence of asthma and allergic rhinitis. The primary exposure variables of interest are the severity and aetiology of the infant viral illness and maternal and familial atopic status and other environmental exposures. Cumulative asthma incidence over time, taking into account loss to follow up, will be used for illustrating incidence data. Incidence of asthma and allergic diseases among the enrolled infant population with viral respiratory illness will be calculated by dividing the number of incident asthma cases by the person time of follow up. A Kaplan–Meier plot of cumulative incidence over time, taking into account loss to follow up, will be used for illustrating incidence data. Incidence rates will be calculated by dividing the patient population into quartiles/quintiles of bronchiolitis severity scores. The adjusted risk of asthma and allergic rhinitis with bronchiolitis severity will be evaluated using the Cox proportional hazard regression model.

To assess the relationship between biomarker concentrations and increased risk of infant bronchiolitis, and early childhood asthma outcomes, we will conduct a nested case–control study. Geometric means of urinary biomarker concentrations for those who develop and do not develop asthma will be calculated separately and compared using the paired *t*-test. The association between these biomarkers and the risk of asthma and allergic rhinitis will be assessed using odds ratios and their corresponding 95% confidence intervals from adjusted logistic regression models. The potential confounders that will be considered in multivariable analyses will include demographic and exposure characteristics.

RESULTS

Subject recruitment for the TCRI Study occurred over 4 years, and was completed in May 2008. Overall, 9329 visits were screened, representing 7632 unique infants, and 2986 of these infants met study eligibility requirements. From the 2986 eligible infants, 674 infants and their biological mothers were enrolled (Fig. 1). Among the 2312 subjects who were available during the recruitment periods, the major reasons for non-response were refusal (22%), insufficient time/

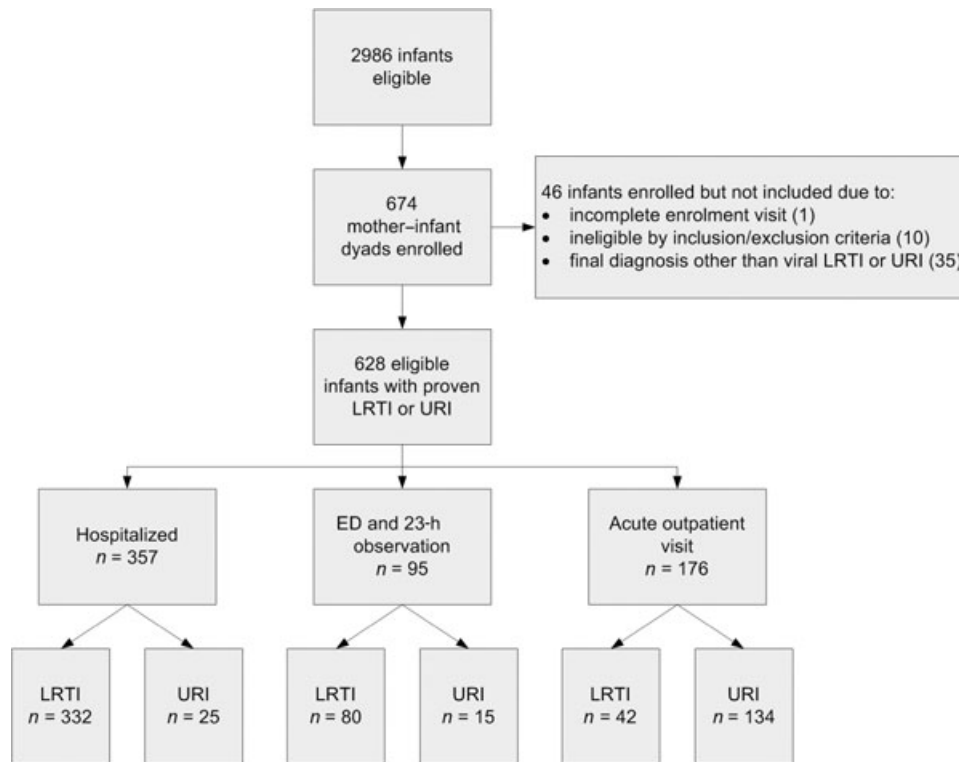


Figure 1 Flow diagram of participants through the screening, enrolment and initial analysis phases of the Tennessee Children's Respiratory Initiative. LRTI, lower respiratory tract infections; URI, upper respiratory tract infection.

unwilling to stay for the visit (outpatients) (39%), conflict with or already enrolled in another study (20%), and other (18%) (includes language barrier, mother/guardian not present, previously enrolled and other miscellaneous). In 99.9% of the cohort, the nasal/throat swab was obtained, and in 79% of the hospitalized infants one spot urine sample was obtained at hospital admission. Weekly enrolment into the cohort is depicted in Figure 2.

DISCUSSION

The TCRI is a large and comprehensive prospective epidemiologic study of mothers and their biologic children enrolled during infancy with a clinically significant LRTI or URI who are being followed through early childhood. This study will provide important information about the role of infant respiratory viral infection severity, aetiology, biomarkers and predictors important in the development of early childhood asthma and allergic rhinitis. The TCRI is additionally unique because it is designed in parallel with a large retrospective birth cohort of over 95 000 mother-infant dyads with similar objectives to investigate the role of respiratory viral infection severity and aetiology in the development of asthma.

As evidence suggests that the development of asthma may result in part from respiratory viral infection during infancy, which has a predilection for infecting, destroying and/or in some way biologically

altering lower airway epithelium, this study will help to delineate whether the severity of that infection and other early-life events impact the risk of asthma and allergic disease development in later childhood.³ Despite a high attack rate of developing asthma following viral bronchiolitis, the majority of children who have infant bronchiolitis do not develop childhood asthma. Thus while viral respiratory infections may alter lung physiology and target the inflammatory response to the lower airway, this may only occur during a vulnerable time period during development of the immune system or lung, or in the presence of other risk factors. This developmental component may further reflect important gene-environment interactions that regulate both short- and long-term airway physiological alterations that manifest themselves clinically as childhood asthma. Efforts to determine and define the role of these factors, including disease severity, maternal atopy and other environmental exposures, such as second-hand smoke, to asthma pathogenesis are the focus and goal of the TCRI.

Several limitations of this study should be noted. First, the study sample was not randomly selected from the general population, but instead was recruited from a single hospital and clinic-based setting, the Vanderbilt Children's Hospital. While Vanderbilt hospitalizations represent greater than 90% of Davidson County/Nashville infant hospitalizations, it represents a smaller proportion of emergency department visits (51%), and likely even fewer

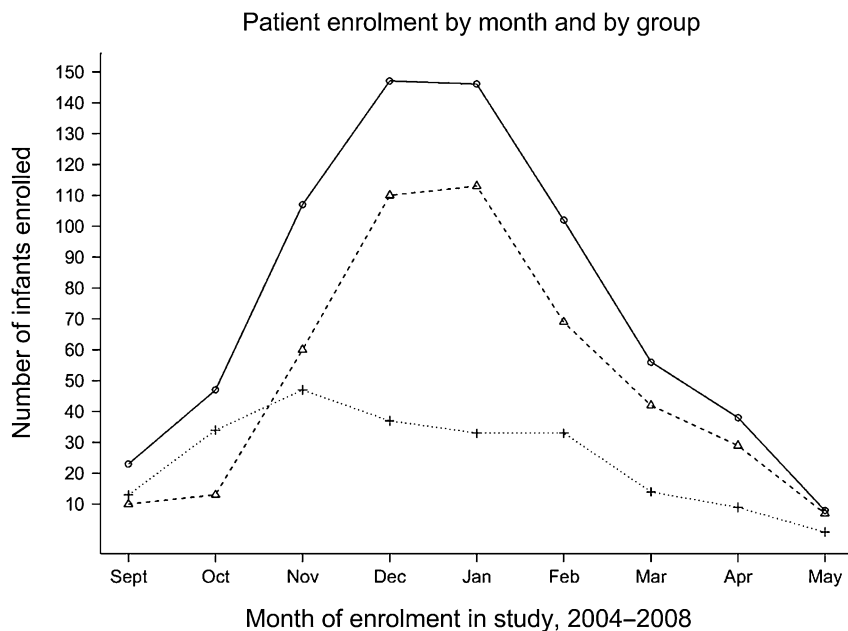


Figure 2 Weekly enrolment during the September through May enrolment periods of the Tennessee Children's Respiratory Initiative, 2004–2008. (—○—) all patients. (—△—) inpatients. (··+··) outpatients.

paediatric acute care visits.² The relatively low participation rate among eligible subjects is multifactorial and the result of the long-term nature of the study, the lack of study personnel to enrol all eligible subjects, as well as lower willingness among outpatients to extend their visit in order to participate in the study. While this impacts the demographics and exposures of the study population, and thus generalizability of our study results, it should not impact the findings of the role of infant viral infection on the outcomes of interest. Next, while airway hyperreactivity is not assessed in making the diagnosis of childhood asthma, the identification of incident asthma cases will take into consideration the positive response to a validated written questionnaire that has been compared with bronchial provocation testing.^{6,8} While such an ascertainment strategy might result in the underdiagnosis of asthma, it is unlikely to result in false positive diagnoses during the sixth year of life. Finally, as with many studies, where all eligible participants were approached for participation, difficulty was encountered in follow up of those currently age-eligible for follow up. Strategies to address this include study personnel doing a significant number of follow-up visits at the subject's home, and shipping follow-up materials and requests to paediatricians of subjects who have moved from the region.

Future reports from this cohort will help to clarify the complex relationship between infant respiratory viral infection severity, aetiology, atopic predisposition, and the development of early childhood asthma and other atopic diseases. Ultimately, this study, along with the companion TABS cohort, has the potential to provide new approaches to identify infants at high risk of developing early childhood asthma and allergic diseases, as well as provide important information that may contribute to the development of prevention strategies.

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