



The Canadian Healthy Infant Longitudinal Development (CHILD) Study: examining developmental origins of allergy and asthma

Padmaja Subbarao,¹ Sonia S Anand,² Allan B Becker,³ A Dean Befus,⁴ Michael Brauer,⁵ Jeffrey R Brook,⁶ Judah A Denburg,² Kent T HayGlass,³ Michael S Kobor,⁵ Tobias R Kollmann,⁵ Anita L Kozyrskyj,⁴ W Y Wendy Lou,⁷ Piushkumar J Mandhane,⁴ Gregory E Miller,⁸ Theo J Moraes,¹ Peter D Pare,⁵ James A Scott,⁷ Tim K Takaro,⁹ Stuart E Turvey,⁵ Joanne M Duncan,² Diana L Lefebvre,² Malcolm R Sears,² the CHILD Study investigators

► Additional material is published online only. To view please visit the journal online (<http://dx.doi.org/10.1136/thoraxjnl-2015-207246>).

¹Department of Pediatrics, Hospital for Sick Children and University of Toronto, Toronto, Canada

²McMaster University, Hamilton, Canada

³University of Manitoba, Winnipeg, Canada

⁴University of Alberta, Edmonton, Canada

⁵University of British Columbia, Vancouver, Canada

⁶Environment Canada, Toronto, Canada

⁷University of Toronto, Toronto, Canada

⁸Northwestern University, Chicago, USA

⁹Simon Fraser University, Vancouver, Canada

Correspondence to

Dr Padmaja Subbarao, Department of Pediatrics, Hospital for Sick Children, 555 University Avenue, Toronto ON, M5G 1X8, USA; padmaja.subbarao@sickkids.ca

Received 28 April 2015

Accepted 13 May 2015

Published Online First

11 June 2015



CrossMark

To cite: Subbarao P, Anand SS, Becker AB, et al. *Thorax* 2015;**70**:998–1000.

ABSTRACT

The Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort study recruited 3624 pregnant women, most partners and 3542 eligible offspring. We hypothesise that early life physical and psychosocial environments, immunological, physiological, nutritional, hormonal and metabolic influences interact with genetics influencing allergic diseases, including asthma. Environmental and biological sampling, innate and adaptive immune responses, gene expression, DNA methylation, gut microbiome and nutrition studies complement repeated environmental and clinical assessments to age 5. This rich data set, linking prenatal and postnatal environments, diverse biological samples and rigorous phenotyping, will inform early developmental pathways to allergy, asthma and other chronic inflammatory diseases.

INTRODUCTION

Determining the origins of allergy and asthma has become an urgent research need. Developed countries face a high burden of allergic diseases and asthma, impacting families, healthcare systems and economies, while allergy and asthma are now also increasing in the developing world. Although family history of allergy or asthma is a risk factor, many children with asthma do not have a positive family history, suggesting that environmental influences are critical, acting either independently or by epigenetic mechanisms.

The Developmental Origins of Health and Disease (DOHaD) hypothesis postulates that environmental exposures influence developmental pathways during critical periods of prenatal life and postnatal life, inducing permanent changes leading to altered disease susceptibility. Indoor and outdoor air pollutants, microbes, viral infections, maternal nutrition, infant feeding practices and psychosocial environments interact with genes and may exert their influence on asthma and allergy susceptibility via epigenetic mechanisms. The Canadian Healthy Infant Longitudinal Development (CHILD) Study is designed to address gaps in understanding complex gene–environment interactions during pregnancy

and early childhood¹ and provides a platform for study of atopic disease and other non-communicable diseases (NCDs) with early life origins.

METHODS

The cohort

Pregnant mothers in their second or third trimester were recruited from the general population in four communities across Canada (Vancouver, Edmonton, Manitoba (Winnipeg, Morden, Winkler) and Toronto) where local Research Ethics Boards approved the study. Inclusion and exclusion criteria for mothers and infants are in online supplementary table E1.

Two planned a priori substudies focused on psychological and infant and preschool lung function determinants and did not require full cohort participation.

Assessments

A timetable of assessments from pregnancy to 5 years is in online supplementary table E2.

Questionnaires (see online supplementary tables E2 and E3). Environmental, psychosocial, nutrition and health questionnaires are administered during pregnancy and at 3, 6, 12, 18, 24, 30 months, 3, 4 and 5 years.

Environment. Household exposures are assessed repeatedly from recruitment to age 5 years. In addition to periodic questionnaires beginning at recruitment, a detailed walk-through assessment of the home was conducted when the child was 3–4 months old and included dust sampling for allergens and pollutants, along with collection of breast milk, child's urine, nasal swab and stool. Repeated biospecimens provide additional exposure biomarker information. Outdoor air pollution exposures are assessed using Land Use Regression models, residential history and time–activity patterns.

Psychosocial assessments. Maternal psychosocial characteristics assessed by self-report during pregnancy, post partum and at 1 year included socioeconomic status, stress, depressive symptoms and social relationships.

Nutrition. A Food Frequency Questionnaire (FFQ) is administered during pregnancy and at



regular intervals for the child during the first 5 years.

Birth. The duration of labour, mode of delivery, anthropometrics, medication including antibiotics, and maternal or fetal complications were obtained from hospital records.

Biological samples

Blood samples. Whole blood, serum and plasma samples were obtained from all consenting mothers and fathers.² Child blood was drawn at birth (cord blood), 1 year and 5 years. Cord blood from 785 infants has been prepared for studies of innate immunity and haemopoietic progenitor biomarkers.

Urine samples. Child urine was collected at 3 months, 1, 3 and 5 years, divided into aliquots, frozen at -80°C and were then stored in liquid nitrogen.

Nasal samples. Nasal swabs (Copan Diagnostics, Corona, California, USA) were collected at 3 months and 1 year in all children, with additional swabs taken during acute illnesses in a subgroup, separated into aliquots, frozen at -80°C and were then stored in liquid nitrogen.

Stool samples. Meconium was collected from infants at birth, and stool at 3 months and 1 year, divided into aliquots, frozen at -80°C and were then stored in liquid nitrogen.

Breast milk. Ten millilitres of breast milk was collected at the home visit, divided into aliquots, frozen at -80°C and then stored in liquid nitrogen.

Clinic assessments

At ages 1, 3 and 5 years, questions from the International Study of Asthma and Allergies in Childhood (ISAAC) were completed by the parent. At each assessment, the child is examined for evidence of atopic dermatitis, allergic rhinitis and asthma. Allergy skin prick tests and general anthropometrics are performed at all assessments. Blood pressure, waist circumference and skin-fold thickness are measured at 3 and 5 years. Spirometry is performed in all children at age 5 years.

Outcomes

The *primary outcome* of the CHILD Study is expert physician diagnosed asthma at age 5 years (see online supplementary figure E1). Secondary outcomes include preschool wheezing, atopic sensitisation, atopic dermatitis, allergic rhinitis and food allergy. Permission was obtained to link with provincial administrative databases enabling studies of healthcare utilisation.

RESULTS

Recruitment

A total of 3624 families were recruited between 2008 and 2012. Gestational age at recruitment ranged from 6 to 39 weeks, mean 26.7 weeks and SD 6.3 (table 1, see online supplementary figure E2). Families who became ineligible after recruitment were excluded (miscarriage (15), prematurity <35 weeks (44), multiple births (5), pregnancy resulting from in vitro fertilisation (3) or complications, abnormalities or fetal death (15)) resulting in 3542 infants for the study. Study retention is 92% at age 1 year.

Parents

Mothers completed a health questionnaire during pregnancy (n=3475), spirometry (3012), allergy skin testing (3073) and provided blood during pregnancy or at 1 year (3369). Of 2893 fathers recruited, 2841 have completed a health questionnaire, 2499 completed spirometry, 2519 underwent allergy skin testing and 2358 provided blood.

Family demographics

In over 25% of families, one or both parents were not of white Caucasian ethnicity (table 1). Just over half, 1868 of 3426 (54.5%), of the index children were firstborns; the mean ages of mother and father were 32.3 and 33.8 years, respectively. Overall, 760 of 3424 mothers (22.2%) and 547 of 3000 fathers (18.2%) reported a personal history of asthma, 2832 of 3425 (82.7%) and 2094 of 3000 (69.7%), respectively, reported 'allergies' and 1768 of 3073 (57.5%) and 1657 of 2432 (68.1%) were objectively atopic by skin prick testing. Among mothers, 890 of 3229 (27.6%) had ever smoked, while 185 of 3423 (5.4%) reported smoking in pregnancy; 373 of 2847 (13.1%) of fathers currently smoked. Significant differences observed among centres in housing characteristics have been described fully elsewhere.³

Infant health outcomes

The prevalence of caesarean section varied among centres (18.2–31.6%, mean 25.6%) (see online supplementary table E4). Cord blood was obtained in 75.1% of deliveries. The average birth weight was 3448 g (SD 482) and length 51.4 cm (SD 2.5); 52% were males. Exclusive breast feeding at 3 months varied by site from 52% to 68%. At 3 months, 1821 of 3111 infants (58.5%) were exclusively breastfed as were 371 of 2679 (13.9% of those with data) at 6 months.

DISCUSSION

Allergic diseases have reached epidemic proportions in middle-income to high-income countries, with a parallel increase in many chronic NCDs in the latter part of the 20th

Table 1 Baseline demographics of parents and families enrolled in the Canadian Healthy Infant Longitudinal Development (CHILD) Study by site

Family demographics		
Mean gestational age in weeks at enrolment (SD)	26.7 (6.3)	
Older siblings in the home (%)	48.3	
Parental demographics		
	Maternal	Paternal
Mean age in years (SD)	32.3 (4.7)	33.8 (5.6)
Reported ethnicity		
White Caucasian	2532 (72.9)	2536 (73.7)
South East Asian	428 (12.3)	343 (10.0)
South Asian	91 (2.6)	106 (3.1)
First Nations	177 (5.1)	145 (4.2)
Black	77 (2.2)	110 (3.2)
Other	161 (4.6)	174 (5.1)
Unknown*	6 (0.2)	29 (0.8)
Education		
High school or less	295 (8.7)	502 (14.7)
College or university	2443 (72.2)	2151 (63.1)
Postgraduate education	647 (19.1)	756 (22.2)
Health status		
Reported asthma	760/3424 (22.2)	547/3000 (18.2)
Any reported allergy	2832/3425 (82.7)	2094/3000 (69.7)
Atopic by skin testing	1768/3073 (57.5)	1657/2432 (68.1)†
Gestational diabetes	115/3475 (3.3)	N/A

Data are presented as n (%) or as n/N (%) with N being the total sample available for that variable. Sample sizes differ for some characteristics due to differences in missing values.

*Those responding that they did not know their ethnicity are classified as 'Unknown'.

†Skin test data apply only to participating fathers.

century. In 2011, for only the second time in its history, the United Nations called a special General Assembly on health describing NCDs as a global epidemic. Asthma, one of the most common NCDs worldwide, is the earliest presenting chronic disease of childhood and the leading cause of childhood morbidity as measured by hospitalisations and school absences.

Lifestyle and environmental factors, including household and outdoor air pollution exposures, diet, physical activity and stress, appear to play important roles in the increase of these chronic diseases. Exposures occurring in utero and in infancy may be critical to the development of immune responses which influence the onset of these complex, chronic diseases, but how these exposures interact to promote allergic disorders versus immune homeostasis without clinical symptoms remains largely speculative.

This Canadian birth cohort study was initiated to study the development of allergy and asthma and designed with a focus on environmental assessments of infants and parents. The 3542 infants and families are predominantly from urban centres; over 80% of the Canadian population is urban. The recruited population is multicultural and ethnically varied, and children represent mixed ethnic populations to a greater degree than historical birth cohorts. The value of this cohort is enhanced by extensive phenotyping of both children and parents, characterisation of their environments and an extensive repository of biological samples.²

A registry⁴ documented over 46 Canadian birth cohorts, comprising some 950 000 individuals, of which CHILD is the only cohort focused on environmental effects on development of allergies and asthma in the general population. The National Institutes of Health and the European Commission research group 'Mechanisms of the Development of Allergy' (MeDALL) co-sponsored a workshop to harness knowledge generated across 130 birth cohorts initiated across many countries within the last 30 years, which have gathered data on asthma.⁵ However, few (<12) encompass the breadth (eg, microbiome to psychosocial assessments) and depth (eg, infant lung function to innate immune phenotyping) of the CHILD cohort. We are actively participating in efforts to create standardised data definitions in conjunction with international initiatives, using standardised questionnaires and transparent data dictionaries to increase the power of such collaborations.

CONCLUSIONS

The CHILD cohort of 3542 eligible children followed to age 5 years with repeated surveys of environmental and other exposures, linking detailed prenatal and postnatal environments, a diversity of biological and environmental samples and careful assessment of developing clinical phenotypes will foster examination of relationships relevant to the development of allergy, asthma and other chronic NCDs with origins in childhood.

Acknowledgements We are grateful to all the families who are participating in this study, and the whole CHILD Study team, which includes interviewers, nurses, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers and receptionists. Thanks to Chinthanie Ramasundarahettige for her careful work with data analysis and presentation. We also acknowledge the generosity of ALK-Abello, Mississauga, ON, Canada, in supplying all allergens for the study, and Lincoln Diagnostics Inc., Decatur, IL USA, for supplying the Duotip-Test II devices and skin testing kits.

Collaborators *Scientific Advisory Committee:* A Scientific Advisory Committee (Paul M O'Byrne (Chair), Fernando D Martinez, Mark E Raizenne, Felix A Ratjen, Peter D Sly, Erika von Mutius) provided valuable advice at start-up and has continued interacting with the Study leadership, contributing their experience in scientific studies, including longitudinal birth cohort studies in USA, Australia and Europe. *CHILD Study investigators:* Sears MR (Director), McMaster University; Subbarao P (co-Director), The Hospital for Sick Children; Allen R, Simon Fraser University; Anand SS, McMaster University; Becker AB, University of Manitoba; Befus AD, University of Alberta; Brauer M, University of British Columbia; Brook JR, University of Toronto; Chen E, Northwestern University, Chicago; Cyr M, McMaster University; Daley D, University of British Columbia; Dell S, The Hospital for Sick Children; Denburg JA, McMaster University; Elliott S, University of Waterloo; Grasemann H, The Hospital for Sick Children; HayGlass K, University of Manitoba; Hegele R, The Hospital for Sick Children; Holness DL, University of Toronto; Lou WYW, University of Toronto; Kobor MS, University of British Columbia; Kollmann TR, University of British Columbia; Kozyrskij AL, University of Alberta; Laprise C, Université du Québec à Chicoutimi; Larché M, McMaster University; Macri J, McMaster University; Mandhane PM, University of Alberta; Miller G, Northwestern University, Chicago; Moqbel R (deceased), University of Manitoba; Moraes T, The Hospital for Sick Children; Paré PD, University of British Columbia; Ramsey C, University of Manitoba; Ratjen F, The Hospital for Sick Children; Sandford A, University of British Columbia; Scott JA, University of Toronto; Scott J, University of Toronto; Silverman F, University of Toronto; Takaro T, Simon Fraser University; Tang P, University of British Columbia; Tebbutt S, University of British Columbia; To T, The Hospital for Sick Children; Turvey SE, University of British Columbia.

Contributors This paper has been prepared by PS and MRS on behalf of the list of authors who have all contributed to its development and approved it for submission. PS and MRS serve as guarantors for the contents of this paper.

Funding The Canadian Institutes of Health Research (CIHR; AEC85761) and the Allergy, Genes and Environment (Allergen) Network of Centres of Excellence provided core funding for the CHILD Study. Support has also been provided by Health Canada, Environment Canada, Canada Mortgage and Housing Corporation, the Sick Children's Hospital Foundation, Don & Debbie Morrison, the Silver Thread Foundation and the Childhood Asthma Foundation.

Competing interests None declared.

Ethics approval Hamilton Regional Research Ethics Board.

Provenance and peer review Not commissioned; internally peer reviewed.

REFERENCES

- 1 Subbarao P, Becker A, Brook JR, *et al.* Epidemiology of asthma: risk factors for development. *Expert Rev Clin Immunol* 2009;5:77–95.
- 2 Moraes TJ, Lefebvre DL, Chooniedass R, *et al.* The Canadian Healthy Infant Longitudinal Development birth cohort study: biological samples and biobanking. *Paediatr Perinat Epidemiol* 2015;29:84–92.
- 3 Takaro TK, Scott JA, Allen RW, *et al.* The Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort study: assessment of environmental exposures. *J Expo Sci Environ Epidemiol*. Published Online First: 25 Mar 2015. doi:10.1038/jes.2015.7.
- 4 Joly MP, Boivin M, Junker A, *et al.* An inventory of Canadian pregnancy and birth cohort studies: research in progress. *BMC Pregnancy Childbirth* 2012;12:117.
- 5 Bousquet J, Gern JE, Martinez FD, *et al.* Birth cohorts in asthma and allergic diseases: report of a NIAID/NHLBI/MeDALL joint workshop. *J Allergy Clin Immunol* 2014;133:1535–46.

ON LINE SUPPLEMENT:

The majority of the methods and detailed assessments for the CHILD study are described in this supplement.

METHODS:

The cohort:

Recruitment: We recruited pregnant mothers from the general population in four provinces of Canada: British Columbia (Vancouver, urban), Alberta (Edmonton, urban), Manitoba (Winnipeg, urban; Morden and Winkler, rural) and Ontario (Toronto, urban). Given the potential for first trimester miscarriages, recruitment was directed to the second trimester, especially targeting 18 weeks gestation when most pregnant women attend regional health centers for ultrasound. Paternal participation was encouraged but not mandatory. Each centre obtained local Research Ethics Board approval for the study, and each participating parent gave signed informed consent.

Study Population: We excluded infants in whom other factors could confound the development of wheezing, such as prematurity (≤ 35 weeks), respiratory distress syndrome, and *in vitro* fertilization involving *ex-utero* manipulation of the ovum, since potential environmental effects and epigenetic changes in these embryos could not be assessed¹. Children expected to spend less than 80% of their time in the primary home were excluded due to inability to model their exposures.

In advance of the main study enrollment we recruited a vanguard sample to allow us time to evaluate our recruitment and retention methods. The main study recruitment was initiated 6 months after the vanguard. This design allows us to continually assess our methods prior to the general cohort visits.

Vanguard cohort: We initially recruited approximately 50 families in each centre, and evaluated ease of recruitment, representativeness, understanding of questionnaires, sample collection and acceptance and comfort with procedures. Following minor revisions to protocols and questionnaires, recruitment of the general cohort began six months later. These Vanguard families remain integral to the CHILD cohort as they continue to inform the age 1, 3 and 5 year visit methodology.

Sub-cohorts: Two sub-studies which did not require participation of the whole cohort were planned *a priori*. In Toronto, families were invited to participate in additional tests of infant lung function and more intensive viral studies, while in Vancouver, a sub-study examined cord blood immunological responses and epigenetic changes in relation to more detailed information on parental psychosocial measures and

stress. Further sub-studies were added after the initial grant application was funded. In Edmonton, families were invited to participate in a study that examined early childhood sleep and sleep disorders². Studies examining the infant gut microbiome and its influence on immunological and clinical outcomes likewise involve sub-cohorts within the overall CHILD Study³.

Assessments:

Development of Questionnaires and Standardized Operating Procedures (SOPs):

Questionnaires:

Data were obtained through environmental, psychosocial, nutritional and health questionnaires administered at recruitment, at 36 weeks gestation (psychosocial only), and at 3, 6, 12, 18, 24, 30 months, 3, 4, and 5 years. Currently questionnaires are available for electronic entry enabling parental completion at home. Data are managed using a secure electronic data capture and management system (HealthDiary Inc., Toronto, Canada) with encryption and automatic backup. Questionnaires are checked for completeness at each centre, and further checked for logic and coding errors during data entry; missing or incomplete data are queried.

The following detailed questionnaires were conducted:

- 1. Environmental Assessments:** Household exposures are assessed by questionnaires repeated from recruitment to age 5 years, as reported elsewhere⁴. Exposure to traffic related air pollutants is based on residential location and city-specific Land Use Regression modeling⁵⁻⁷. The child's time-activity patterns are tracked and second-hand tobacco smoke exposure assessed by repeated questionnaires and cotinine measurements in infant urine. A home visit conducted when the child was 3-4 months old included a detailed walk-through assessment of the home including basement and garage if applicable, and dust sampling for multiple allergens and pollutants, along with collection of biomarker samples (breast milk, the child's urine, nasal swab and stool).
- 2. Psychosocial assessments:** Maternal psychosocial characteristics assessed by self-report during pregnancy included socioeconomic status (SES), life stress, depressive symptoms, and social relationships; family income and wealth, parental education and occupation; mother's subjective social standing; exposure to chronic difficulties and acute events in major life

domains (family, work, health, school) [19,20], and global perceived stress⁸ (see online supplement). Maternal depressive symptoms were assessed by the Center for Epidemiologic Studies – Depression Scale⁹, social support by the Interpersonal Support Evaluation List¹⁰, and partner relationships with the Dyadic Adjustment Scale¹¹. These assessments are repeated annually. In the Vancouver sub-cohort, in-depth assessments of stressors and depression utilized the UCLA Life Stress Interview – Adult Version¹², while the Depression module of the Structured Clinical Interview for DSM-IV yielded data on history and presence of major depressive episodes¹³; these were repeated when the child reached one year of age.

3. **Nutritional Assessments:** A Food Frequency Questionnaire (FFQ) developed and validated by nutritional epidemiologists at the Fred Hutchinson Cancer Research Center¹⁴ was modified to include Canadian ethnic food choices, and the database developed by the University of Minnesota Nutrition Data Systems for Research for data entry and nutrient analysis^{15, 16} was similarly updated for Canadian food products.
4. **Birth:** Duration of labour, mode of delivery, child anthropometrics, medication use including antibiotics, and maternal or fetal complications were obtained from hospital records.

Biological Samples:

Maternal, paternal and infant biological samples have been divided into multiple aliquots and maintained in liquid nitrogen for assays for biomarkers of exposure and clinical outcomes¹⁷. Each aliquot is individually identified and tracked through HealthDiary.

1. **Blood samples:** Whole blood, serum and plasma samples were obtained from all mothers and most fathers. Child blood was drawn at birth (cord blood), 1 year and 5 years. Using carefully developed and rigorously assessed SOPs, blood samples from the children were prepared for aliquots of serum, plasma, DNA, RNA and frozen/retrievable mononuclear cell populations. The latter were chosen for several reasons, including their clinical relevance and common use in immunological assays. Moreover, mononuclear cells are devoid of multinucleate granulocytes, which might confound subsequent epigenetic and transcriptomic analyses. Cord blood from 785 infants has been prepared for studies of innate immunity by stimulating samples with a range of innate stimuli prior to cryopreservation^{18, 19}, while over 200 cord blood samples have been collected for studies of hemopoietic progenitor biomarkers.

2. **Urine samples:** Child urine was collected at 3 months, 1, 3 and 5 years, divided into 6 aliquots, frozen at -80C then stored in liquid nitrogen.
3. **Nasal samples:** Nasal swabs were collected at 3 months and 1 year, by gently rubbing a nasal turbinate with a flocked swab (Copan Diagnostics, Corona California) which was then placed in universal transport media. In the laboratory, the sample was separated into 6 aliquots, frozen at -80C then stored in liquid nitrogen.
4. **Stool samples:** Meconium was collected from infants at birth, and stool at 3 months and 1 year, divided into aliquots, frozen at -80C then stored in liquid nitrogen.
5. **Breast Milk:** On the day of the home visit, mothers collected 10mL of breast milk (by hand expression or pump) into a previously supplied container and refrigerated it until transported on ice to the central laboratory facility, where it was vortexed, divided into aliquots, frozen at -80C then stored in liquid nitrogen.

The following provides detailed methods for specific investigations:

ENVIRONMENTAL EXPOSURE MEASUREMENTS:

Full details of environmental assessments in the CHILd Study are available in another publication⁴. A comprehensive baseline questionnaire (during pregnancy) with detailed updates at 3 months, 1, 3, and 5 years of age record address, housing structure, function, condition, maintenance and cleaning habits, presence and use of attached garage, renovations, source and extent of dampness, visible mold, new furnishings, appliance and household cleaner emissions, personal care products, presence and type of air conditioning and cleaning, and conditions of use. Questions related to the child's and families' activities include time spent in different rooms in the house and indoors vs. outdoors, time in transit, mode of transport, frequency/duration of visits to daycare, indoor pools and exposure to smoke. Shorter update questionnaires at 6 and 18 months, 2, 2.5 and 4 years of age focus on child time-activity and major changes including renovations. When the infant was 3-4 months old, research staff trained by Canada Mortgage and Housing Corporation assessors and the Environmental Scientific Working Group visited the home. This enabled measurement of geographical coordinates (GPS) for assigning air pollution and Traffic-Related Air Pollution (TRAP) exposure, evaluation of the structure, function and exposure sources within the home with emphasis on cleanliness and cleanability, furnishings, ventilation (air-conditioning, heating, cooling), potential for moisture build-up, microbial and chemical contaminant

burdens, basement conditions and potential for air exchange with the outside and with attached garages. Collectively, the breadth of exposures characterized will enable development of novel exposure indices for integrating multiple oxidative and/or inflammatory exposures to better capture total risk posed by the physical environment.

Household exposures: Questionnaires included animal exposures (dogs, cats, other pets, observed pests) at multiple time points. Dampness (water leaks), mold and household chemical use were also assessed by repeated questionnaires, and visual inspection of the home. House and child's bedroom dust was systematically sampled with a specially designed vacuum collection system, weighed, sieved and stored. A subset of the fine dust has been analyzed for endotoxin and β -glucans to support questionnaire validation and application in regards to dampness and mold. A range of organic chemicals are also being quantified (e.g., phthalates, PAHs) at Environment Canada laboratories. We will analyze dust for dog, cat, house dust mite and other allergens to complement skin prick testing conducted at 1, 3 and 5 years.

Second hand smoke (SHS): Smoking in or near the home was recorded by questionnaire during pregnancy and early childhood, including smoking outside the home, near an open window or in the garage. Exposure to SHS in other locations (e.g., daycare) is also assessed by the questionnaire. Urinary cotinine measurements at 3-4 months of age are used to validate exposure.

Outdoor air pollution exposures: We have characterized both large scale patterns and the small-scale spatial gradients in TRAP and other combustion-related pollutants within each city. With home coordinates, and addresses of locations the children frequently visit (e.g., daycare) we can assign a time-weighted air pollutant exposure. Land use regression (LUR) models have been developed for each city to predict exposure to TRAP⁵⁻⁷. Individual air pollution exposure assessment is based on land use regression models, residential history and time-activity patterns²⁰. The National Air Pollutant Surveillance Program (NAPS) monitoring data and Environment Canada air quality model objective analyses are available to capture the larger scale patterns. Time activity data, obtained frequently during the first 3 years and annually after age 3, provides weighting to these two levels of outdoor exposures.

Exposure Biomarkers: Maternal and child serum, breast milk, cord blood, meconium, nasal swabs, stool and urine samples have been acquired and bio-banked for multiple uses, including analysis for exposure biomarkers¹⁷. Urine collected at 3 months, 1 and 3 years in a subset of the cohort has been analyzed to

assess exposure to SHS and phthalate plasticizers to support key Federal Government programs. These data are then used to validate the questionnaire and improve how the multiple questions pertaining to SHS and phthalate exposure are weighted to assign exposure. Eight phthalate metabolites have been measured (AXYS laboratories, Victoria, BC) for a matched subset (n=900) using 3, 12 and 36 month urine samples. Similarly, cotinine and hydroxy-cotinine have been measured through collaboration with the US Centers for Disease Control laboratories (Atlanta).

Exposure to other children: This will be assessed through annual time-activity logs that include a daycare questionnaire, age when the child first started daycare, age when the child left daycare, and the number of hours per week spent in daycare.

PSYCHOSOCIAL ASSESSMENTS:

Four maternal psychosocial characteristics were assessed by self-report during pregnancy: socioeconomic status, life stress, depressive symptoms, and social relationships (**Table E5**). To capture the multi-dimensional nature of SES, we collected indicators of family income and wealth, parental education and occupation, and mother's subjective social standing. Life stress was assessed by capturing mothers' exposure to chronic difficulties and acute events in major life domains (family, work, health, school)^{21, 22}. Mothers' global perceived stress was assessed as the extent to which they found life to be unmanageable and uncontrollable⁸; the severity of recent maternal depressive symptoms by the Center For Epidemiologic Studies – Depression Scale⁹ and social support by the Interpersonal Support Evaluation List, which measures the extent to which individuals perceive themselves as having 3 distinct kinds of social support available - tangible, belonging, and emotional support¹⁰. The quality of each mother's relationship with her romantic partner (if present) was assessed with the Dyadic Adjustment Scale¹¹. These assessments were repeated annually following the child's birth.

More in-depth assessments of stressors and depression were conducted in the Vancouver sub-cohort, by administering the UCLA Life Stress Interview – Adult Version¹². This objectively catalogs exposure to acute and chronic stressors over the past 12 months in multiple life domains, including relationships with partners, relatives, and friends; difficulties at work and/or school; and the health of the respondent and his/her family. The Depression module of the Structured Clinical Interview for DSM-IV yielded data on history and presence of major depressive episodes¹³. These interviews were repeated when the child reached one year of age.

CLINICAL ASSESSMENTS:

At ages 1, 3 and 5 years, questionnaires validated in the International Study of Asthma and Allergies in Childhood (ISAAC)²³ are completed by the parent. The child is examined for evidence of atopic dermatitis, rhinitis or asthma. General anthropometrics (weight, height, head circumference), blood pressure, waist circumference and skin-fold thickness (sub-scapular and mid upper arm using Holtain calipers) are measured at 3 and 5 years.

SKIN ALLERGY TESTING AND INTERPRETATION:

Standardized inhalant allergens and common food allergens (ALK Abello Pharmaceuticals Canada) are applied to the children by trained staff using Duotip II devices (Lincoln Diagnostics Canada) at 1, 3 and 5 years, and once only for mothers and participating fathers. Individual wheal sizes for allergens or positive (histamine 0.1%) and negative (glycerin) controls are determined by the mean of the longest diameter and its perpendicular.

Children at 1 year: Epicutaneous skin tests were administered to each infant at approximately 1 year of age for six inhalant allergens (*Alternaria tenius*, Cat Hair standardized, Dog Epithelium, House Dust Mites (*Dermatophagoides pteronyssinus*, *D. farinae*), German cockroach) and four food allergens (whole cow's milk, egg white, soybean, peanut). The same batch of each ALK allergen was used at all sites. Histamine 1mg/mL and Glycerin were used as positive and negative controls.

Children at 3 years: In addition to all allergens tested at age 1 year, the following seven ALK allergens were added to the panel: *Cladosporium*, *Penicillium* mixed, *A.fumigatus*, Midwest trees, grass mix, weed mix, and mixed ragweed.

Children at 5 years: The same 17 allergens employed at 3 years were repeated at 5 years.

Parents: To define parental atopy, mothers and participating fathers were tested with a panel of 14 ALK allergens: *Alternaria tenius*, Cat Hair standardized, Dog Epithelium, *D. pteronyssinus*, *D. farinae*, German cockroach, *Cladosporium*, *Penicillium* mixed, *A. fumigatus*, Midwest trees, grass mix, weed mix, mixed ragweed and peanut.

Interpretation of skin test results:

On the day of skin testing, research assistants ascertained and recorded what medications and/or herbal supplements were taken by participants. Participants reporting use of antihistamines during the seven days prior to the date of skin prick test were excluded. The wheal responses were measured at 10 minutes for histamine and 15 minutes for allergens. We averaged the maximum diameter and its orthogonal, and defined a positive response as a wheal diameter ≥ 2 mm greater than the response to the negative control, following precedent in previous epidemiological studies²⁴ and evidence that even a 1mm wheal is significant in epidemiological research²⁵. We included all subjects with a positive response to histamine and no response to glycerin, or those with one or more positive responses (≥ 2 mm) to any allergen, even if there was a weak or no response to histamine. Subjects with a positive response to one or more allergens but also a response to the negative control were included with adjustment for the negative control (subtraction of the mean wheal diameter of the negative control from each positive test wheal diameter). In some cases specific tests were omitted (e.g. some families declined infant peanut testing) and these specific allergens responses were recorded as missing but all other data from that participant were included. If allergy tests were conducted by non-CHILD physicians, results were incorporated into the dataset for the individual in order to avoid false negative assignments for atopy. Subjects with no response to histamine and no response to any allergen were excluded, as were subjects with dermatographism, when the response to the negative control was as large as or larger than any other response. If the negative control was not tested, but some allergen tests yielded negative results, we assumed the negative control result was "0" and retained all tests²⁰. Results are expressed both as a dichotomous variable based on ≥ 2 mm response and also as an atopy index, the sum of all wheal sizes to all tested allergens, both after adjusting for any response to the negative control²⁶.

LUNG FUNCTION ASSESSMENTS:

Toronto participants were invited to participate in a sub-study examining lung growth trajectory. As such, parents were invited to an infant pulmonary function visit at 3, 12 and 18 months. Traditional infant and preschool lung function assessment techniques have been combined with methods to assess lung ventilation inhomogeneity and airway inflammation. Spirometry is performed in all 3 year olds in Toronto, and at all sites in 5 year olds.

Infant Pulmonary function tests: Assessments were performed at birth, at 3, 12 and/or 18 months, and will be performed at 3 and 5 years. Tidal breathing parameters were measured using Exhalyzer D, Eco Medics AG, Switzerland within the first 12-48 hours of life during natural sleep. Lung ventilation inhomogeneity is measured in infancy, and at 3 and 5 years. Lung Clearance Index (LCI), Moment ratios,

and FRC are measured according to ATS/ERS guidelines²⁷ for Multiple Breath Washout measurement using a respiratory mass spectrometer (AMIS 2000, Odense, Denmark) or commercially available N2 washout system Exhalyzer D, Eco Medics AG, Switzerland and custom software for offline analysis. Exhaled nitric oxide in infancy and at 5 years is measured using tidal breathing technique CLD 88 Exhalyzer D, Eco Medics AG, Switzerland and a facemask and following ATS/ERS guidelines for exhaled nitric oxide measurement. A minimum of 2 epochs were obtained. The average of the two trials is reported. Forced expiratory volumes and flows (FVC, FEV0.5, FEF25-75, and FEF75) were measured on two occasions in infancy between 3 and 18 months using the RVRTC technique according to ATS/ERS guidelines for raised volume forced expirations in infants²⁸ via the nSpire® Infant Pulmonary Lab (IPL). Measurement of forced expiratory flows and volumes were repeated 10 minutes after administration of 4 puffs of albuterol. Lung function outcomes are reported as the single best pre- and post albuterol maneuvers with the highest sum of FVC and FEF25-75.

Raised volume RTC with bronchodilator responsiveness is carried out using standardized methodology at 3 months, 1 year and 2 years of age in the Collins BabyBox. Children at age 3 and 5 years will use sRaw by plethysmography, and perform spirometry where possible with bronchodilator response.

Preschool Spirometry (FVC, FEV0.75, FEF25-75, and FEF75) at 3 years in the Toronto sub-cohort is measured according to ATS/ERS guidelines for preschool lung function via a spirometer (nidd EasyOne spirometer, nidd Medical Technologies ©, USA) and using age-appropriate incentive screens. Lung function outcomes are reported as the single best pre- and post-salbutamol maneuvers with the highest sum of FVC and FEF25-75. The same methodology will be repeated in the entire cohort at age 5 years, and FEV₁ also recorded if possible.

INFECTIONS:

Infections were assessed by questionnaire in the full cohort and routine nasal swabs were assessed at 3 months and 12 months. In addition, parents in the Toronto sub-cohort were invited to call a hotline if their infant experienced symptoms of a lower respiratory tract infection [LRTI] in the first year of a life. With each episode, a standardized symptom questionnaire (Respiratory Illness Score Card from the URECA study)²⁹ was administered. If the infant had LRTI symptoms of sufficient severity (>score of 5) a nurse visited the home, performed a formal clinical respiratory assessment and obtained a nasal swab for viruses. For scores <5, the questionnaire was repeated until there was a resolution in symptoms or a viral swab was obtained.

Nasal swab: A nasal swab sample was collected in all children at 3 months and 1 year, by gently rubbing a nasal turbinate with a flocked swab. The swab was then placed in universal transport media at room temperature (Copan Diagnostics, Corona California), and later separated into 3 aliquots and frozen at -80°C. Subsequently, nucleic acid will be extracted from two aliquots using the bioMérieux MiniMag extractor (bioMérieux, Marcy-l'Etoile, France). The ID-TagT respiratory viral panel (RVP) (Tm Bioscience Corporation, Toronto, Ontario) bead-based microarray method will be used to detect the presence of common respiratory viruses (influenza A and B, parainfluenza types 1-4, RSV, enterovirus/rhinovirus, coronaviruses, metapneumovirus and adenovirus) in the first aliquot. For samples which are negative by the RVP, the second aliquot will be tested on the Virochip microarray which tests for all known viruses simultaneously. The third aliquot will be used for virus-specific PCR tests to confirm the results of the RVP or Virochip.

The upper respiratory tract microbiome (viral and bacterial components) will be assessed through a combination of metagenomics and 16S rRNA deep amplicon sequencing, examining the differences in the microbiome between children as well as changes between 3 months and 1 year. The microbiome analysis will include children reporting acute lower respiratory tract infections to determine correlations between the microbiome and infections.

DATA LINKAGE:

Parents gave permission to link provincial prescription and health-care databases with study data both for themselves and the child, allowing, for example, comprehensive measurement of early life exposures to antibiotics and vaccinations. Linkage with these databases utilizes encrypted health identification numbers.

Outcomes:

Primary Outcome:

The primary outcome of the CHILD Study is asthma at age 5 years, determined using the current gold-standard definition, physician diagnosis by a Pediatric Consultant with expertise in asthma. Standardized histories and clinical assessments will be used to determine:

1. **Definite asthma:** consistent and typical clinical history of symptoms and objective evidence of bronchodilator response, with $\geq 12\%$ change in Forced Expiratory Volume in 1 sec (FEV₁), further subdivided into atopic (defined by one or more allergen skin test wheal with median diameter $\geq 2\text{mm}$ larger than the negative control, or the presence of overt atopic dermatitis) and non-atopic asthma.

2. **Possible asthma:** consistent symptoms but no bronchodilator response, or no clinical history of symptoms but with bronchodilator response.
3. **No asthma:** No history of symptoms and no bronchodilator response.

Secondary Outcomes:

Preschool wheezing: Extensive health questionnaires, administered at 3 and 6 months, then repeated every 6 months from 1 year to 3 years, then annually to 5 years, include questions on wheeze frequency, triggers, and asthma diagnosis. The number and severity of exacerbations are noted, and a detailed and comprehensive medication history elicited. Symptom clusters will be identified, and used in conjunction with family data and objective measurements to determine a modified Asthma Predictive Index (mAPI)³⁰.

Atopic sensitization: After adjusting for any response to the negative control) for each child, atopy is characterized by both a cumulative index (sum of all positive allergen wheal sizes)²⁶ and dichotomously, defined by 1 or more positive SPT with mean wheal diameter ≥ 2 mm above the negative control response^{24, 31}.

Atopic dermatitis: Atopic dermatitis is assessed by the clinical history of recurrent or persistent skin rashes in classical locations²³, and verified on clinical examination. Atopic dermatitis is defined by British Association of Dermatologists criteria³²: an itchy skin condition (or report of scratching or rubbing in a child), plus three or more of the following: a history of itchiness in skin creases such as folds of the elbows, behind the knees, fronts of ankles, or around the neck (or the cheeks in children under 4 years); a history of asthma or hay fever (or history of atopic disease in a first degree relative in children under 4 years); general dry skin in the past year; visible flexural eczema (or eczema affecting the cheeks or forehead and limbs in children under 4 years); onset in the first 2 years of life.

Allergic Rhinitis: This is defined as persistent rhinorrhea, nasal pruritus, and/or nasal congestion in the absence of an apparent upper respiratory tract infection (as defined by the parent) or triggered by specific exposures (e.g., cat, dog) in the presence of a positive SPT to an inhalant aeroallergen³³.

Food allergy: This is defined as a clinical pattern of acute symptoms, primarily cutaneous such as urticaria, but including anaphylaxis, gastrointestinal or respiratory symptoms, or the presence of allergic eczema which repeatedly flared in relation to food ingestion in a child with a corresponding positive skin test³⁴.

Table E1: Inclusion and exclusion criteria

Inclusion Criteria

Pregnant women aged >18 years (>19 years in Vancouver).

Residential proximity (<50 Km) to participating delivery hospital.

Ability to read, write and speak English.

Willing to donate cord blood.

Planning to deliver at a designated recruitment center participating hospital.

Infants born at or after 35 weeks.

Exclusion Criteria

Children born with major congenital abnormalities or respiratory distress syndrome (RDS).

Expectation of moving away from a recruitment centre within 1 year of recruitment.

Children of multiple births.

Children resulting from *in vitro* fertilization.

Children who will not spend at least 80% of nights in the index home.

Table E2: Timetable of assessments

Age of child	Data collection
Pregnancy (18+ weeks)	Maternal, paternal demographics; paternal spirometry and skin testing; clinical, stress, nutrition and environment questionnaires
Pregnancy (36 weeks)	Maternal stress questionnaires repeated
Birth	Delivery outcomes, cord blood, meconium
3-4 months	Home visit: home assessment, dust sampling; breast-milk; child urine, nasal swab and stool; child health questionnaires, medications Sub-cohorts: infant lung function, detailed stress assessment
6 months	Web-based or mail-out questionnaires: environmental update, child health, nutrition
1 year	Clinic visit: child allergy skin tests, blood, urine, nasal swab and stool; clinical assessment; lung function (Toronto sub-cohort); maternal spirometry and allergy skin tests; detailed environmental questionnaire
18 months	Web-based or mail-out questionnaires: follow-up environmental, health and nutrition
2 years	Web-based or mail-out questionnaires: follow-up environmental, health and nutrition
30 months	Web-based or mail-out questionnaires: follow-up environmental, health and nutrition
3 years	Clinic visit: questionnaires: environmental update, child clinical assessment and allergy skin tests, urine; blood (Manitoba sites only); pre-school lung function (Toronto site only)
4 years	Web-based or mail-out questionnaires: follow-up environmental, health and nutrition
5 years	Clinic visit: questionnaires, child clinical assessment, allergy skin tests, lung function, blood, physician assessment of final outcome

Table E3: Key variables obtained by questionnaire

HOME	MOTHER	FATHER	CHILD
Current residence	Maternal demographics	Paternal demographics	Mode of delivery
Previous residences (12 mo)	Maternal health ³⁵	Paternal health ³⁵	Medications around birth
Changes of residence	Maternal medications	Paternal medications	Sleeping arrangements
Type and age of home	Maternal smoking ³⁵	Paternal smoking ³⁵	Activities outside home
Characteristics of home	Maternal respiratory symptoms ³⁵	Paternal respiratory symptoms ³⁵	Colds and infections ²³
Attached garage	Maternal diagnosed asthma ³⁵	Paternal diagnosed asthma ³⁵	Coughing episodes ²³
Heating and cooling systems	Maternal allergies ³⁵	Paternal allergies ³⁵	Wheezing episodes ²³
Humidifiers	Maternal occupation	Paternal occupation	Medications
Basement/crawl space	Health of other children	Hobbies and activities in home	Food allergy ²³
Water leaks and mold	Health during pregnancy		Atopic dermatitis / eczema ^{23, 32}
Swimming pool, spa	Diet before and in pregnancy		Doctor visits
Renovations	Vitamins and supplements		Hospital/ER visits
Furniture	Prenatal/postnatal maternal stress ^{8, 10, 11, 21, 22}		Breastfeeding
Cooking systems	Socioeconomic status		Introduction of milk, solids
Cleaning habits	Depression module ⁹		Vaccinations
Chemicals used in home	Labor and delivery		Time/activity/locations
Smoking in the home	Post-partum health		Travel times and exposures
Characteristics of bedroom	Post-partum stress ^{8, 10, 11, 21, 22}		Daycare arrangements
Animals in home (pets)	Breastfeeding		
Insects and pests in home	Parenting stress ^{8, 10, 11, 21, 22}		

Table E4: Birth outcomes

Birth outcomes			
Caesarean section	829/3237 (25.6)	Male sex	1770/3380 (52.4)
Cord blood obtained	2544/3388 (75.1)	Discharged with mother	3008/3102 (97.0)
Birth weight (n=3192)	3448 gm, SD 482	Hospital stay > 7 days	41/3063 (1.3)
Birth length (n=2306)	51.4 cm, SD 2.5	Given antibiotics	116/2158 (5.4)
Head circumference (n=2294)	34.6 cm, SD 1.5		

Unless otherwise specified, data are presented as n/N (%) with N being the total sample available for that variable.

Table E5: Psychosocial assessment instruments utilized in the full cohort and the Vancouver sub-cohort

Full cohort						Vancouver sub-cohort
Age	SES	Social Support and Relationship Quality	Centre for Epidemiological Studies Depression Scale	Perceived Stress Scale	Chronic Difficulties and Acute Events	UCLA Life Stress Interview and Structured Clinical Interview from DSM-IV
Recruitment	✓	✓	✓	✓	✓	✓
1 year	✓ *	✓	✓	✓	✓	✓
2 years	✓ *	✓	✓	✓	✓	
3 years	✓ *	✓	✓	✓	✓	
4 years	✓ *	✓	✓	✓	✓	
5 years	✓ *	✓	✓	✓	✓	

* Objective and subjective SES measures are administered at all times, but a modified version is used subsequent to the first administration given the stability of some indicators.

FIGURE E1: CHILD Study overview

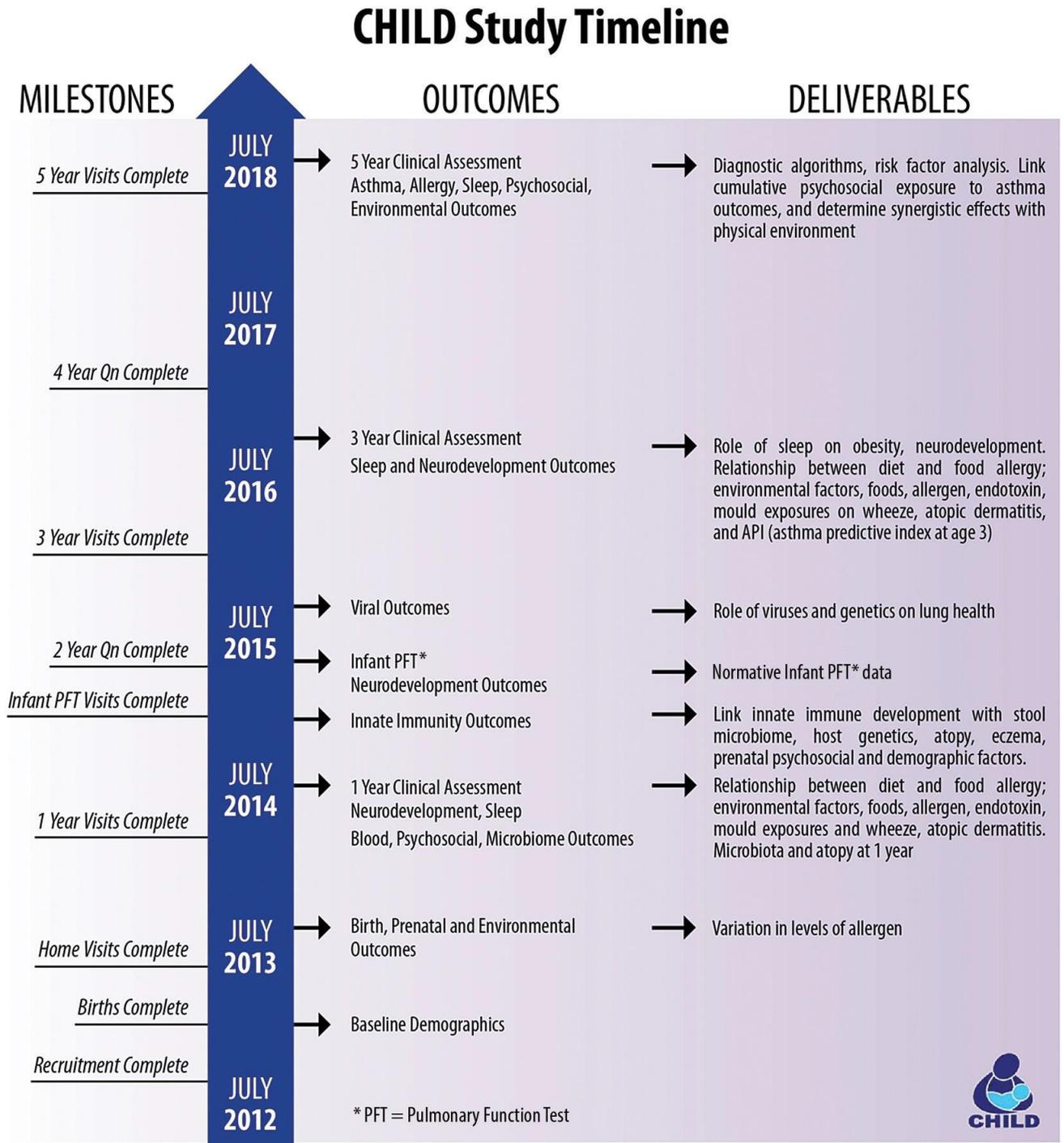
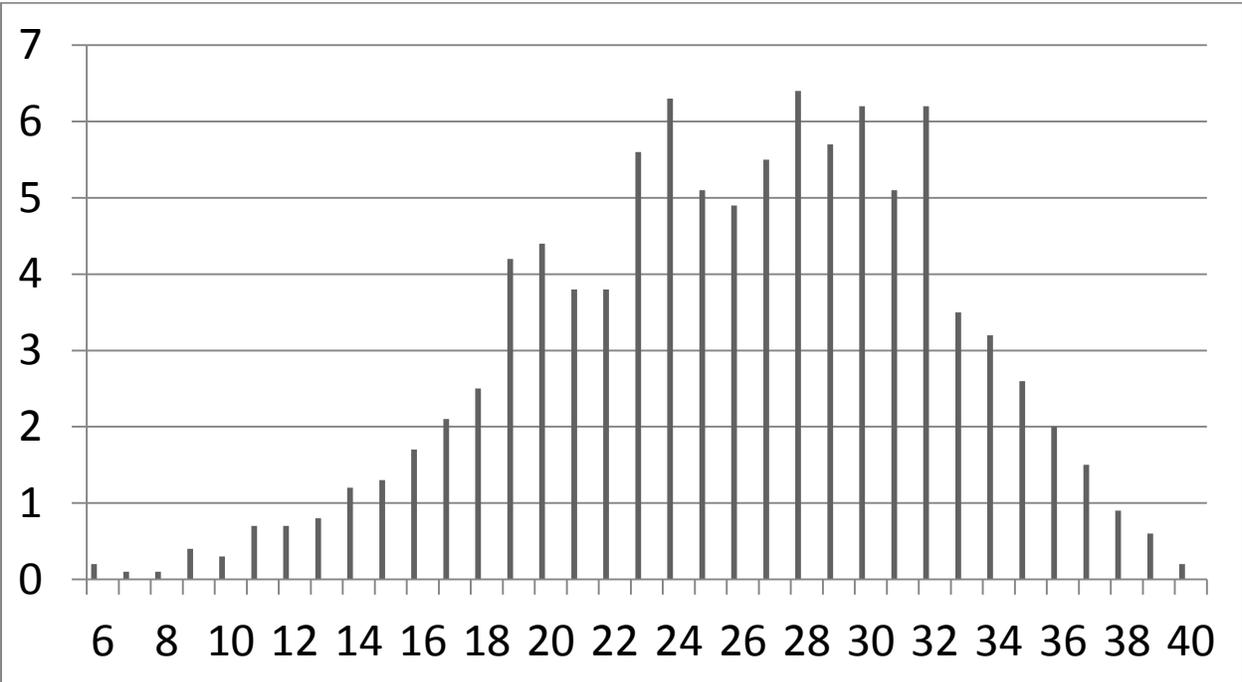


Figure E2: Recruitment by gestational age



Percent of total cohort recruited by week of gestational age, all centres combined. X-axis; week of gestation. Y-axis; percent of total cohort.

REFERENCES

1. Shufaro Y, Laufer N. Epigenetic concerns in assisted reproduction: update and critical review of the current literature. *Fertil Steril* 2013; 99:605-6.
2. Lau A, Mariasine J, Sears MR, Subbarao P, Becker A, Turvey S, et al. Risk Factors For Sleep Disordered Breathing In The First Year Of Life: A Sub-Study Within The Canadian Healthy Infant Longitudinal Development (CHILD) Study. *Am J Respir Crit Care Med* 2014:A1805-A.
3. Azad MB, Konya T, Maughan H, Guttman DS, Field CJ, Chari RS, et al. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *CMAJ* 2013; 185:385-94.
4. Takaro TK, Scott JA, Allen R, Anand S, Becker A, Befus AD, et al. The Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort study: assessment of environmental exposures. *J Exp Sci Envir Epi* 2015:1-25.
5. Henderson SB, Beckerman B, Jerrett M, Brauer M. Application of land use regression to estimate long-term concentrations of traffic-related nitrogen oxides and fine particulate matter. *Environmental Science and Technology* 2007; 41:2422-8.
6. Allen RW, Amram O, Wheeler AJ, Brauer M. The transferability of NO and NO₂ land use regression models between cities and pollutants. *Atmospheric Environment* 2011; 45:369-78.
7. Jerrett M, Arain MA, Kanaroglou P, Beckerman B, Crouse D, Gilbert NL, et al. Modeling the intraurban variability of ambient traffic pollution in Toronto, Canada. *Journal of Toxicology and Environmental Health - Part A: Current Issues* 2007; 70:200-12.
8. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav* 1983; 24:385-96.
9. Radloff L. The CES-D Scale. *Applied Psychological Measurement* 1977; 1:385-401.
10. Cohen S, Mermelstein R, Kamarck TW, Hoberman H. Measuring the functional components of social support. In: Sarason IG, Sarason BR, editors. *Social support: Theory, research, and application* The Hague, Holland: Martinus Nijhoff; 1985. p. 73-94.
11. Spanier GB. Measuring Dyadic Adjustment: New Scales for Assessing the Quality of Marriage and Similar Dyads. *Journal of Marriage and Family* 1976; 38:15-28.
12. Hammen C. Generation of Stress in the Course of Unipolar Depression. *Journal of Abnormal Psychology* 1991; 100:555-61.
13. First MB, Spitzer RL, Gibbon M, Williams JBW. *Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-patient Edition (SCID-I/NP)*. New York: Biometrics Research, New York State Psychiatric Institute; 2002.
14. Patterson RE, Kristal AR, Tinker LF, Carter RA, Bolton MP, Agurs-Collins T. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. *Ann Epidemiol* 1999; 9:178-87.
15. Sievert YA, Schakel SF, Buzzard IM. Maintenance of a nutrient database for clinical trials. *Controlled Clinical Trials* 1989; 10:416-25.
16. <http://sharedresources.fhcrc.org/content/ffq-sample-booklets>. . 2015.].
17. Moraes TJ, Lefebvre DL, Chooniedass R, Becker AB, Brook JR, Denburg J, et al. The Canadian Healthy Infant Longitudinal Development birth cohort study: Biological samples and biobanking. *Paediatric and Perinatal Epidemiology* 2015; 29:84-92.
18. Blimkie D, Fortuno ES, Yan H, Cho P, Ho K, Turvey SE, et al. Variables to be controlled in the assessment of blood innate immune responses to Toll-like receptor stimulation. *Journal of Immunological Methods* 2011; 366:89-99.
19. Kollmann TR, Levy O, Montgomery RR, Goriely S. Innate Immune Function by Toll-like Receptors: Distinct Responses in Newborns and the Elderly. *Immunity* 2012; 37:771-83.

20. Sbihi H, Allen RW, Becker A, Brook JR, Mandhane P, Scott JA, et al. Perinatal Exposure to Traffic-Related Air Pollution and Atopy at 1 Year of Age in a Multi-Center Canadian Birth Cohort Study. *Environ Health Perspect* 2015.
21. Turner RJ, Avison WR. Status variations in stress exposure: Implications for the interpretation of research on race, socioeconomic status, and gender. *Journal of Health and Social Behavior* 2003; 44:488-505.
22. Wheaton B. *Stress and mental health : contemporary issues and prospects for the future*. New York: Plenum Press; 1994.
23. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. *Lancet* 1998; 351:1225-32.
24. Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. *New England Journal of Medicine* 2003; 349:1414-22.
25. Bousquet PJ, Chatzi L, Jarvis D, Burney P. Assessing skin prick tests reliability in ECRHS-I. *Allergy: European Journal of Allergy and Clinical Immunology* 2008; 63:341-6.
26. Sears MR, Burrows B, Flannery EM, Herbison GP, Holdaway MD. Atopy in childhood. I. Gender and allergen related risks for development of hay fever and asthma. *Clinical and Experimental Allergy* 1993; 23:941-8.
27. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, et al. Consensus statement for inert gas washout measurement using multiple- and singlebreath tests. *European Respiratory Journal* 2013; 41:507-22.
28. ATS/ERS statement: raised volume forced expirations in infants: guidelines for current practice. *Am J Respir Crit Care Med* 2005; 172:1463-71.
29. Wood RA, Bloomberg GR, Kattan M, Conroy K, Sandel MT, Dresen A, et al. Relationships among environmental exposures, cord blood cytokine responses, allergy, and wheeze at 1 year of age in an inner-city birth cohort (Urban Environment and Childhood Asthma study). *J Allergy Clin Immunol* 2011; 127:913-9 e1-6.
30. Chang TS, Lemanske RF, Guilbert TW, Gern JE, Coen MH, Evans MD, et al. Evaluation of the modified asthma predictive index in high-risk preschool children. *Journal of Allergy and Clinical Immunology: In Practice* 2013; 1:152-6.
31. Bousquet J, Gern JE, Martinez FD, Anto JM, Johnson CC, Holt PG, et al. Birth cohorts in asthma and allergic diseases: report of a NIAID/NHLBI/MeDALL joint workshop. *J Allergy Clin Immunol* 2014; 133:1535-46.
32. McHenry PM, Williams HC, Bingham EA, Allen R, Breathnach SM, Hopkins A, et al. Management of atopic eczema. *British Medical Journal* 1995; 310:843-7.
33. Broek JL, Bousquet J, Baena-Cagnani CE, Bonini S, Canonica GW, Casale TB, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines: 2010 Revision. *Journal of Allergy and Clinical Immunology* 2010; 126:466-76.
34. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and management of food allergy in the United States: Summary of the NIAID-sponsored expert panel report. *Journal of Allergy and Clinical Immunology* 2010; 126:1105-18.
35. Variations in the prevalence of respiratory symptoms, self-reported asthma attacks, and use of asthma medication in the European Community Respiratory Health Survey (ECRHS). *Eur Respir J* 1996; 9:687-95.