



The Canadian Healthy Infant Longitudinal Development Birth Cohort Study: Biological Samples and Biobanking

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Abstract

Background: It is hypothesised that complex interactions between genetic and environmental factors give rise to allergy and asthma in childhood. The Canadian Healthy Infant Longitudinal Development (CHILD) study was designed to explore these factors.

Methods: CHILD is a longitudinal, general population birth cohort study following infants from mid-pregnancy to age 5 years. Over this time period, biological samples, questionnaires, clinical measures and environmental data are collected.

Results: A total of 3624 families have been recruited, and many thousands of samples and questionnaires have been collected, annotated, and archived. This report outlines the rationale and methodology for collecting and storing diverse biological samples from parents and children in this study, and the mechanisms for their release for analyses.

Conclusions: The CHILD sample and data repository is a tremendous current and future resource and will provide a wealth of information not only informing studies of asthma and allergy, but also potentially in many other aspects of health relevant for Canadian infants and children.

Keywords: birth cohort, biological samples, asthma, allergy.

The Canadian Healthy Infant Longitudinal Development (CHILD) study is a multi-centre longitudinal, prospective, general population birth cohort study following infants from pregnancy to age 5 years. The

rationale for this study has been described in detail elsewhere.¹ Briefly, the incidence of allergy and asthma is rising worldwide,² and although various theories have been proposed to explain this epidemic, it is not clear what factors are keys. Thus, the prevention of allergy and asthma remains elusive. The Developmental Origins of Health and Disease concept posits that *in utero* and early life events play a critical role in the expression of disease in later years.³ The primary hypothesis of the CHILD study is that

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genetic, environmental, and host factors (*in utero* and post partum) interact in the developing fetus and infant to alter the risk of subsequent asthma and allergy. This hypothesis recognises the complex nature of asthma and allergy development; no single factor leads to allergy or asthma in all individuals. For example, the current paradigm suggests that recurrent environmental exposures such as viral infections and aeroallergen exposure together alter the development of the susceptible host lung and predispose to asthma.⁴ Understanding what factors identify a susceptible individual, what factors constitute a risky exposure, and how these factors relate to the developing infant is the goal of the CHILD study. In that regard, biological samples may provide early markers of disease and inflammation that not only predict subsequent diagnoses but may also shed light on disease mechanisms. The processes involved in the collection and storage of samples are critical to ensure accurate and meaningful results. This manuscript documents which samples are being collected and the methodology involved in order to act as a resource for those performing similar studies, and also to promote collaboration with researchers who see value in the samples collected from this cohort.

Methods

The CHILD study is designed to follow infants from pregnancy to age 5 years with multiple questionnaires, assessments, and biological sample collections (Table 1). Enrolment began in 2008 and closed in 2012 with a total of 3624 pregnant mothers recruited. This report outlines the rationale and methodology associated with the collection and storage of the biological samples and provides a current tally on the sample types and aliquots obtained.

Although the focus of this paper is on biological samples, a strength of the CHILD study is the ability to link biologic measures with other components of the study, including clinical testing, environmental assessments, and questionnaires. Briefly, questionnaires were administered at recruitment, 36 weeks gestation, at 3, 6, 12, 18, 24, 30 months and at 3, 4, and 5 years. In this way, data are obtained related to environmental exposures, psychosocial stresses, nutrition, and general health. In addition, at ages 1, 3, and 5 years, questionnaires validated in the International Study of Asthma and Allergies in Childhood (ISAAC)⁵ are completed by the parent. At age 1, 3,

and 5, the child is examined for evidence of atopic dermatitis, rhinitis, or asthma. Blood pressure, and skin-fold thickness (sub-scapular and upper arm), is measured at 3 and 5 years. 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 3-year-olds in Toronto, and at all sites in 5-year-olds. Skin testing for standardised inhalant allergens and common food allergens are performed by trained staff at 1, 3, and 5 years. Parents also gave permission to link their data, and their child's study data to provincial prescription and health care databases, allowing, for example, comprehensive measurement of early life exposures to antibiotics and vaccinations.

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). Recruitment was directed to the second trimester when most pregnant women attend regional health centres for ultrasound. Inclusion and exclusion criteria for mothers and infants are shown in Table S1. Baseline demographics are shown in Table S2. Infants in whom other factors could confound the development of wheezing were excluded, such as prematurity. *In vitro* fertilisation was excluded because potential environmental effects and epigenetic changes in these embryos could not be assessed.⁶ Children expected to spend less than 80% of their time in the primary home were excluded because of the inability to model their exposures. All mothers and the majority of fathers participated with their infants, but participation of the father was not a mandatory requirement.

Follow-up

Subjects are being followed longitudinally over multiple visits (Table 1) during which numerous questionnaires are administered and biological samples collected. Ultimately, data from the questionnaires and from analyses of biological samples will allow prospective evaluation of the effects of multiple indoor and outdoor environmental exposures; the development of immunological responses; lung function

Table 1. CHILD study schedule from prenatal recruitment to age 5

	18 Weeks	36 Weeks	Birth	3 Months	6 Months	1 Year	1.5 Years	2 Years	2.5 Years	3 Years	4 Years	5 Years
Questionnaires												
Mom profile	x			x		x		x		x	x	x
Mom health	x					x						x
Mom nutrition	x			x		x						x
Mom vitamins	x			x		x						x
Mom medications	x		x	x	x	x						
Mom stress	x			x	x		x	x	x	x	x	x
Mom psychosocial		x										
Mom life stress interview		V				V						
Parenting						x		x		x	x	x
Dad health	x											x
Socioeconomic status	x					x				x	x	x
Child delivery			x									
Child health				x	x	x	x	x	x	x	x	x
Child nutrition				x	x	x	x	x	x	x	x	x
Child medication			x	x	x	x	x	x	x	x	x	x
Child clinical assessment						x				x	x	x
Home environment	x			x	x	x	x	x	x	x	x	x
Home assessment				x								
Food packaging						x				x	x	
Mom skin prick						x						
Mom spirometry						x						
Dad skin prick	x											
Dad spirometry	x											
Child skin prick										x		x
Child eNO			T	T		T	T					x
Child lung function tests				T		T	T			T		x
Cord blood			x									
Mom breast milk												
Mom venous blood	x					x						
Mom/dad buccal swab ^a						x						
Dad venous blood	x											
Child venous blood						x				W		x
Child buccal swab ^a						x						
Child nasal swab				x		x						
Child urine				x		x				x		x
Child stool			x	x		x						
Home dust				x								

^aeNO¹ denotes exhaled nitric oxide. 'T' denotes items collected only at Toronto site (infant lung function). 'V' denotes items collected only at Vancouver site (Mom life stress interview). 'W' denotes items collected only at Winnipeg site (3-year child blood).

^aBuccal swabs collected only when DNA not obtained from blood.

and airway inflammation; and the associations of infections, allergies, socioeconomic factors, stressful environments, genetics, diet, and nutrition, with clinical outcomes. Samples will be used to generate microbiome, metabolome, genetic, and epigenetic data sets to study the role of these factors as well.

Collection and the processing of the samples are described in more detail below corresponding to the time period of collection.

Recruitment prenatal visit

Parents were recruited at routine antenatal obstetrical ultrasound visits, which generally occurred at or after 18 weeks gestation. At other times, parents were recruited for the study at kiosks at community events, malls, and baby shows. Across the sites, approximately 60–85% of recruitment was completed in health care locations. This information, however, was not captured in sufficient detail to be able to determine separate demographic characteristics.

Of the 3624 recruited mothers, 9.8% were recruited ≤ 18 weeks gestation, 25.1% were recruited from 18 to 24 weeks gestation, 34.1% were recruited from 24 to 30 weeks gestation, and 31% were recruited >30 weeks gestation.

Birth

For the specific sites, deliveries occurred in two hospitals in Toronto, three hospitals and a birthing centre in Winnipeg, one hospital in Morden/Winkler (rural MB site), five hospitals in Edmonton, and four hospitals and one birthing centre in Vancouver. All sites have had some home births (total of 71 births). A detailed standard operating procedure was provided for blood collection and all supplies were provided centrally by the National Coordinating Centre (NCC). The NCC project manager trained the coordinators who then provided in-servicing to staff at each of the hospitals. In addition, for each mother, a premade collection kit for cord blood and meconium samples was provided containing a standardised checklist to document the collection dates/times, collection procedure instruction sheet, unique sample labels, and standardised clinical supplies. Once the samples were collected, blood was stored at 2–8°C, and the site staff were notified so that the blood could be transported to the processing laboratory. Sample processing forms were used to collect blood volumes, date/time of blood col-

lection, date/time of delivery of blood to the processing laboratory, date/time for commencement of processing procedures, and date/time for deposition of aliquots in storage.

Cord blood Cord blood was successfully collected at 2547 deliveries (70.3% of recruited mothers) by trained staff directly from an umbilical vein after the cord was clamped and before the placenta was delivered. Documentation was available for 970 subjects detailing why a cord blood sample was not obtained; reasons included staff forgetting to obtain a blood sample, a complicated or emergent delivery, parents electing to use blood for banking, or the blood clotted when collection was attempted. From heparinised cord blood pooled in a 50-mL conical tube, several different samples were obtained (see details for each below and Table 2). The number of aliquots and the prioritisation of outcomes were predetermined by the total blood volume available (see Tables S4 and S5). At the Vancouver and Toronto sites, a portion (2 mL) was used for analysis of innate immune responsiveness (discussed in Online Supplement S1 in more detail). The numbers of aliquots of these cord blood derivatives are shown in Table 3.

Meconium The labour and delivery nurse was instructed to place a liner (3M Tegaderm HP Transparent Film Dressing, 9536HP; 3M St. Paul, MN, USA) in the newborn infant's diaper and to change the liner with the diaper until meconium had been deposited into the liner. The soiled diaper and liner was placed into a collection bag and refrigerated. Study personnel were contacted and processed the sample within 24 h. Stool was aliquoted into four cryovials using stainless steel depyrogenated spatulas. The weight of each aliquot was recorded and samples were stored at -80°C . In total, 2740 meconium samples were processed with the majority (88.1%) contributing sufficient for 4 aliquots.

Home visit

A home visit was conducted when the infant was aged 3–4 months [16.6 ± 4.9 (standard deviation, SD) weeks]. This involved a thorough assessment of the home, completion of questionnaire data, and collection of a variety of samples from the home, mother, and infant including house dust, breast milk, stool, nasal swabs, and urine (Table 1). Research assistants

Table 2. CHILD laboratory cord blood processing – protocols and outputs

Protocol number	Blood volume (mL)	Sample type	Number of cryovials	Volume per cryovial (mL)
1	6	Serum	6	0.5
Protocols below use heparinised blood				
2	2	Innate assay		
(In centres collecting for innate assays)				
		Plate 1: 6 h	8	0.1
		Plate 2: 24 h	10	
		Plate 3: 24 h	N/A	
3	2	Whole blood DNA and protein	4	0.5
4	1	Whole blood RNA	2	0.5
5	4	Whole blood plasma	8	0.5
6		Cord blood mononuclear cell preparation		
7	6	CBMC in RLT buffer	6	0.25
8	20	CBMCs cryopreservation	10	0.5

CBMC, Cord blood mononuclear cells; RLT, RNeasy Lysis Buffer.

undertaking the home visits were trained during a 2-day workshop taught by experienced home inspectors from the Canadian Mortgage and Housing Corporation. To facilitate the assessment, a detailed instructional booklet with sample collection supplies was sent to study participant homes in advance.

House dust To maximise yield of house dust samples, study participants were instructed not to clean or vacuum their floors 1 week prior to the assessment. Dust samples were collected from two areas in the home; the room where the child sleeps (child's bedroom, CB), and another area in the home where the child spent most of his or her time (most-used living area, MULA). The sample collected from the

child's room was a combination of dust collected from the bed mattress and bedroom floor. The bed mattress dust sample was collected after the bed covers and top bed sheet were removed to expose the bottom bed sheet. If space in the bedroom allowed, four squares (0.71 m²) of floor were measured and taped, one in an area where the child was likely to play and three not in direct sunlight. Dust samples were collected by vacuuming in one direction in a straight line making 10 passes until the demarked squares were completed (Sanitaire Canister Vac: Model S3680; Electrolux Bloomington, IL, USA). The reservoir thimble was then removed from the depyrogenated hognose roller dust collection tool (the vacuum head) (Figure S1) and transferred into a glass vial using sterile tweezers. The

Table 3. Aliquots collected from cord blood samples

	Number of aliquots										
	0 (%)	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)	7 (%)	8 (%)	9 (%)	10 (%)
RNA	251 (9.9)	351 (13.8)	1945 (76.4)								
DNA	176 (6.9)	250 (9.8)	71 (2.8)	113 (4.4)	1937 (76.1)						
Serum	295 (11.6)	247 (9.7)	491 (19.3)	738 (29)	598 (23.5)	101 (4)	77 (3)				
Plasma	376 (14.8)	113 (4.4)	113 (4.4)	98 (3.8)	174 (6.8)	72 (2.8)	116 (4.6)	100 (3.9)	1385 (54.4)		
CBMC	513 (20.1)	86 (3.4)	192 (7.5)	140 (5.5)	127 (5)	132 (5.2)	132 (5.2)	98 (3.8)	143 (5.6)	59 (2.3)	925 (36.3)

Table 4. Aliquots of dust collected from the home

	Number of aliquots							
	0 (%)	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)	7 (%)
CB	0 (0)	0 (0)	548 (17)	81 (2.5)	123 (3.8)	97 (3)	79 (2.4)	2304 (71.3)
MULA	1 (0)	0 (0)	611 (19)	62 (1.9)	68 (2.1)	66 (2)	47 (1.5)	2366 (73.5)

glass vial was stored at room temperature; dust was then aliquoted into seven cryovials with one containing 50 mg, four cryovials containing 25 mg, and the rest of the dust was split evenly into cryovials 6 and 7.

CB samples were collected from 3232 subjects and MULA samples from 3221 subjects. The majority (>70%) of collections resulted in storage of 7 aliquots (Table 4) of each CB and MULA samples. To ensure quality control of dust samples, every 12th home visit included a NIST dust control (National Institute of Standards and Technology, Gaithersburg, MD, USA) (See Online Supplement S1 for more details).

Breast milk The day prior to the scheduled home visit, mothers were asked to hand express breast milk on two occasions, prior to and after feeding their infant. If the mother was unable to hand express breast milk, a breast pump was suggested as an alternate option. The brand and model of the breast pump used was recorded. These samples were date and time stamped. A minimum of 10 mL was collected in a 60-mL sterile polypropylene collection jar and kept in the participant's refrigerator until pick up. The sample was transported to the CHILD laboratory on ice in a cooler, aliquoted into six 2-mL cryovials, and frozen at -80°C degrees. Of the 2598 subjects (71.6% of total cohort) that contributed a breast milk sample, 96.3% (2501) provide enough volume for 6 aliquots.

Infant stool The child's stool was also collected at the home visit. Tegaderm liners for the diaper were included in the mailed package. Recognising that infant patterns vary, participants were asked to place the liner in their child's diaper at least 3 days prior to the home visit to ensure that a sample was gathered before the visit. The diaper containing the stool sample was kept in a sealed plastic bag in the refrigerator. If multiple samples were obtained, the most recent stool sample was accepted and transferred to the laboratory on ice in a cooler. The sample was aliquoted into four 2-mL cryovials using a stainless

steel depyrogenated spatula. The total weight was recorded and samples were frozen at -80°C . Samples from 2973 subjects (82% of total cohort) have been collected with 94.1% of subjects providing 4 aliquots.

Nasal swab Nasal swabs were collected from the child using a sterile nasal swab tipped with flocked nylon fiber (COPAN swab # 56750CS01; Copan Diagnostics Inc., Murrieta, CA, USA). Maintaining aseptic technique, research assistants gently inserted the swab 3 mm into the infant's nose and rotated the swab to obtain samples. Samples were taken from both nares with the same swab. The swab was then placed in 3 mL of pink universal transport medium (COPAN MINI UTM CA353C) and the vial placed in a cooler for transport to the laboratory. Within 8 h of collection, the sample was vortexed for 15 s and then aliquoted to six cryovials (0.5 mL each). Of the 3207 subjects (88.5% of total cohort) that contributed a 3-month nasal swab sample, 84% provided 6 aliquots and 13.6% resulted in 5 stored aliquots.

Urine To collect a relatively clean infant urine sample, at the beginning of the home visit, the research assistant placed a Tegaderm liner with three cotton pads on top in the middle of a new diaper (in the area most likely to become wet with urine). Initial studies in the Vanguard cohort demonstrated that the Tegaderm liner did not contaminate the sample with phthalates or phthalate metabolites (see Online Supplement S1). The diaper was examined at the end of the visit (approximately 2 h later), and taken to the family washroom. Using gloves, the research assistant removed the plunger from a 20-mL sterile syringe, placed the soaked cotton pads inside the syringe, replaced the plunger, and squeezed the cotton pads to directly aliquot urine into six 2-mL cryovials. One drop of the residual sample was placed on a refractometer for a specific gravity measurement. The cryovials were transported on ice in a cooler to the laboratory and stored at -80°C within 8 h of

collection; 2816 subjects (77.7% of total cohort) have contributed urine samples at the home visit with 89.6%, providing enough volume for 6 aliquots to be stored. As with the dust samples, urine samples were also controlled with collection of a quality control standard (see Online Supplement S1).

One-year visit

The 1-year visit [1.1 ± 0.17 (SD) years] occurred in an outpatient clinic in a hospital or clinic setting. At this visit, maternal blood, infant blood, stool, nasal swab, and urine were collected, using the methods described above.

Maternal blood Maternal blood was collected and processed for aliquots of serum, plasma, and whole blood with the protocol used at the prenatal visit; 2799 mothers (77.2 % of total cohort) provided a maternal blood sample at the 1-year visit. The number of aliquots obtained is shown in Table S6.

Infant blood A 25 gauge to $23 \times \frac{3}{4}$ ' gauge butterfly needle was used to collect approximately 6 mL of blood. On average 3.0 mL of heparinised blood and 2.25 mL of non-heparinised blood has been collected from 2082 subjects to date (57.4 % of total cohort). Green (heparinised) and red vacutainers were processed at each CHILD study laboratory utilising protocols similar to those used for cord blood. Heparinised blood was used for whole blood (2 aliquots of 0.25 or 0.5 mL), plasma (2 aliquots of 0.25 or 0.5 mL), peripheral blood mononuclear cells (PBMCs) (2 aliquots from 1.5 to 3 mL whole blood) and, in centres where samples were prepared for analysis of innate immune responsiveness, for innate immunity plates (see Online Supplement S1). Nonheparinised blood was used to collect serum (2 aliquots of 0.3 mL), and cell pellets were stored for DNA analysis. The numbers of aliquots of blood obtained from the 2082 infants at 1 year are shown in Table 5.

Saliva/buccal swabs If a cord blood sample or a 1-year blood sample was not provided, a buccal swab was offered as an opportunity to contribute a sample for DNA processing at 1 year. Buccal swabs were also collected from parents who did not contribute blood (see Online Supplement S1).

Table 5. Aliquots collected from 1-year infant blood sample

	Number of aliquots		
	0 (%)	1 (%)	2 (%)
DNA	149 (7.2)	1933 (92.8)	
Serum	498 (23.9)	135 (6.5)	1449 (69.6)
Whole blood	130 (6.2)	621 (29.8)	1331 (63.9)
Plasma	561 (26.9)	571 (27.4)	950 (45.6)
PBMC	730 (35.1)	280 (13.4)	1072 (51.5)

Three-year visit

The 3-year visit ($3.0 + / - 0.17$ [SD] years) occurred in an outpatient clinic in a hospital or clinic setting. Thus far less than half the cohort has passed this age; an additional 1700 3-year visits are expected in the next year. At the 3-year visit, only infant urine is collected, either directly (as per the 5 year visit, see below), or indirectly with cotton pads (as at 3 months and 1 year). To date 538 subjects have contributed a 3-year urine sample and 98.5% of these collections resulted in 6 aliquots being stored.

Five-year visit

The 5-year visit will occur in an outpatient clinic in a hospital or clinic setting. At time of writing, samples have just begun to be collected from members of the cohort that have reached this age. Child blood and urine will be collected at this visit. Venous blood from the child will be collected using a $21 \times \frac{3}{4}$ ' gauge to 25 gauge butterfly needle. A total of 16 mL of blood will be collected: 5 mL in a red vacutainer for serum (and cell pellet), 10 mL in a green vacutainer for whole blood, plasma, and PBMCs and 1 mL in a lavender vacutainer for complete blood count (CBC) analysis. Urine will be collected in a urine commode specimen container (Fisher 22363149). Prior to aliquoting and freezing ($1 \text{ mL} \times 6$ aliquots), a refractometer will be used for a specific gravity measurement as above.

Storage, logistics, and quality control

All samples have been and will continue to be collected and processed at each local recruitment site and stored at -80°C and/or in liquid nitrogen prior to shipping to the central storage site in Hamilton, ON. To track samples, a two-dimensional bar code is fitted to each sample aliquot to provide a unique laboratory

number and link the aliquot to other data associated with the study subject. HealthDiary (HealthDiary Inc., Toronto, ON, Canada) is a multi-centre, electronic data capture and clinical data management system that is used to track CHILD samples (see Online Supplement S1).

Study organisation

The CHILD study involves over 40 investigators, with a director and an executive committee made up of the principal investigator, the four recruitment site leaders (co-principal investigators), and six additional investigators, representing various scientific fields of interest. Several scientific working groups are advisory to the executive and have informed the design of CHILD and continue to provide input into the collection of relevant data and samples from subjects to ensure that CHILD addresses its primary hypothesis as well as the multiple secondary and multidisciplinary hypotheses that have been enunciated.¹ Part of the executive and working group mandate is to generate and prioritise additional secondary hypotheses to ensure that samples are used appropriately towards informing the primary hypothesis.

Access to samples

A Biological Samples Committee (BSC), a sub-committee of CHILD study co-investigators, advises the Executive Committee on releasing CHILD samples for analysis. The BSC mandate is to ensure that proposed studies align with the goals and priorities of the CHILD study and that samples are analysed efficiently by rigorous state-of-the-art methods, to maximise outcomes and minimise sample wastage. Sample requests are submitted on a request template providing information on five domains (see Appendix S1 in online supplement S1). The request is received by the NCC, reviewed by the principal investigator or designate for completeness and then forwarded to the BSC for evaluation. Within 2 weeks, the assessments are returned to the chair of the BSC. The assessments are collated and, if necessary, a teleconference is convened to discuss and clarify concerns and formulate a final recommendation, which is submitted to the principal investigator and Executive Committee for a final decision regarding approval of the request. This entire process is ideally completed within 4 weeks. If samples are released, the applicant and principal

investigator co-sign a Material Transfer Agreement on the terms of sample release, data sharing, reporting, and dissemination.

Summary

The CHILD study is an exciting, ambitious, and transformative endeavour charged to uncover key determinants of early life development of asthma and allergy. This project involves the collection, processing, and storage of thousands of biological samples from thousands of subjects. These samples are linked to extensive questionnaire data and clinical outcomes. This sample and data repository is a tremendous resource and will provide a wealth of information not only relevant to asthma and allergy, but also to many other aspects of health of Canadian children.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Online Supplement S1. Supplementary tables and methods as indicated in the text are found in the online supplement.

Appendix S1. CHILD sample request form.

Figure S1. Illustration of hognose roller dust collection tool. Rendered illustration of hognose roller vacuum dust collection tool exploded (left) and assembled (right). The body of the device was fabricated in aluminium, and the wheels and connectors in teflon to permit heat sterilisation and depyrogenation between collection sites and prevent marring of hard flooring surfaces. When assembled, each of the two barrels in the central tube of the collector houses a nylon dust collection thimble (DUSTSTREAM, Indoor Biotechnologies, Charlottesville, VA, USA). The 'double-barrelled' collector provides a twofold increase in filter collection area over the manufacturer's standard collector, reducing the influence of dust loading on collector back pressure. For collection, the device is affixed to the nozzle of a household vacuum cleaner.

Table S1. Inclusion and exclusion criteria.

Table S2. Baseline demographics of cohort.

Table S3. Aliquots (0.5 mL) of blood samples collected at prenatal visit.

Table S4. Cord blood sample prioritisation based on volume available (in centres not collecting for innate immunity assays).

Table S5. Cord blood sample prioritisation based on volume available (in centres collecting for innate immunity assays).

Table S6. Aliquots collected from 1-year maternal blood sample.

Online Supplement:

Supplemental Methodology:

Parental blood:

Parental blood was collected as serum, plasma and whole blood by routine venipuncture using a 22-gauge needle. Blood collection was coordinated with a scheduled prenatal obstetrical visit, when feasible. When possible, paternal blood was collected as well as maternal blood. A 10 mL red vacutainer was used for serum (4 aliquots of 0.5 mL) and a 10 mL green (sodium heparin) vacutainer used for whole blood (2 aliquots of 0.5 mL) and plasma (6 aliquots of 0.5 mL). A total of 3,120 mothers had blood samples processed at recruitment, and for the majority (>95%), the maximum number of aliquots was obtained (**Table S3**). Fewer fathers had samples processed at this time point (2,342), although further paternal samples are expected. As with the maternal samples, most fathers contributed the maximum number of aliquots (**Table S3**). All aliquots were frozen and stored at -80°C in sterile cryopreservation vials (cryovials, Corning #430488).

Cord Blood:

Collection of 3 mL in an EDTA (Ethylenediaminetetraacetic acid) tube for a complete blood count (CBC) analysis of cord blood was completed for 1,315 subjects. The CBC was processed by individual site hospital labs. An average of 24 mL of heparinized cord blood and 5 mL of non-heparinized cord blood was obtained per subject. Up to 6 mL of the non heparinized blood was used to provide serum. The remainder of the available cord blood, collected in up to 5 heparinized vacutainers, was pooled into one 50 mL conical tube. The blood was kept at room

temperature (inside a Styrofoam container) until processing at each site CHILD study laboratory.

Time of delivery, cord blood collection time and time elapsed until blood processing was recorded; 43% of cord bloods were processed within 24 hours.

Up to four 0.5 mL aliquots of whole cord blood were frozen for future DNA, protein or whole blood analyses. RNA was stored from 1 mL of blood mixed with 2.6 mL of RNAlater (Ambion, #AM7024) and aliquoted into cryovials. The remaining blood volume was recorded and the blood centrifuged at 500 x g for 10 minutes to obtain a plasma supernatant. Up to eight 0.5 mL aliquots of plasma were collected in cryovials. The cell pellet was resuspended in PBS (volume based on initial pre-plasma extraction volume) and then cord blood mononuclear cells (CBMCs) were extracted with Ficoll (Ficoll-Paque PLUS, GE Healthcare) using the manufacturer's standard protocol. Up to 6 mL of the CBMC solution (approximately 2.5×10^6 cells per mL) was pelleted and resuspended in Buffer RLT-Plus (600 μ L per mL, Qiagen) for nucleic acid isolation. The cryovials were then directly frozen at -80°C. The remaining CBMCs were pelleted, resuspended in a pre-chilled mixture of newborn calf serum (NCS, virus and mycoplasma free, Invitrogen #26010) and RPMI 1640 culture medium containing 2 mM L-glutamine (RPMI, Gibco #72400-047) and then added to freezing media (50% NCS and 10% Dimethyl Sulfoxide (DMSO; Hybrimax, Sigma, # D2650) in RPMI). RPMI, DMSO and NCS were sterile filtered and endotoxin tested. The final solution was transferred to 10 cryovials in 0.5 mL aliquots with approximately 5×10^6 cells per cryovial. These cryovials were placed in a Mr. Frosty (Nalgene, # 5100-0001) in a -80°C freezer allowing the cells to freeze gradually. After 2 to 24 hours, cryovials were moved from the Mr. Frosty to liquid nitrogen storage.

Innate immune stimulation of cord blood cells:

For innate immune assessment, 2 mL of heparinized whole cord blood was mixed with 2 mL of pre-warmed (37°C) RPMI (i.e. diluted by 50%). This diluted blood was added to specific wells (200 µL per well) in two premade 96-well round bottom plates (Corning #3799). These wells contained innate immune stimuli in duplicate wells.¹ The stimuli used included: no stimulus as a negative control; a TLR1/2 stimulus (pam3CSK4, EMC Microcollections, Tübingen, Germany) at 0.1 and 1 µg/mL, a TLR4 stimulus (lipopolysaccharide from *E. coli* O111:B4, InvivoGen, San Diego, CA) at 10 and 100 ng/mL and a TLR7/8 stimulus (R848, InvivoGen) at 1 and 10 µM. The first innate plate also contained the secretion inhibitor Brefeldin to permit flow cytometric assessment of intracellular cytokines at a later point; this plate was incubated for 6 hours at 37°C and 5% CO₂. Cells were detached from each well following a short incubation with EDTA (2mM final for 15 minutes), and the mixture from each well transferred to a cryovial containing 1400 µL of BDFACSLyse (Becton Dickinson) and stored in liquid nitrogen. The second plate (without secretion inhibitor) was incubated for 24 hours, centrifuged (250 x g for 5 minutes) to allow harvesting of 100 µL of the supernatant, which was frozen in a fresh 96-well plate at -80°C. The cell pellet in the original plate was also detached and stored in BDFACSLyse as described above. Thus each blood sample collected for innate analysis provided up to 3 outputs. Among those from whom cord blood was obtained, there were 777 subjects with a 6 hour incubation for intracellular cytokine analysis, 785 subjects with a 24 hour incubation for cell pellet analysis and 778 subjects with a 24 hour incubation for supernatant analysis.

Home Visit:*Dust control:*

Using a stainless steel depyrogenated spatula, 200 mg of NIST dust was placed into a glass vial collection bottle and capped before being taken to the home. At the home visit, while vacuuming up the first dust sample the prefilled glass NIST dust bottle was uncapped for 5 minutes and placed in the same area as where the sample was being collected in order to mimic sample handling. Control samples were labeled as controls and processed/stored with samples from study subjects.

Urine control:

These standards consisted of cryovials of water provided from a central site frozen and stored at each local site. At every 12th collection, a cryovial of the water standard was brought to the home and allowed to thaw. While the urine was being collected, the water standard cryovial was opened so as to have the field blank cryovials open during the time of urine aliquoting. Once the urine collection was completed, the cap was replaced on the water standard and the label 'field blank' placed on the cryovial.

In the initial Vanguard cohort study, tegaderm was not used in the urine collection procedure. However, this Vanguard identified that only small urine volumes could be collected due to the superior wicking ability of disposable diapers. As a result, a small pilot study was conducted by the NCC using disposable diapers with and without tegaderm and mock urine samples (distilled water prepared by a central laboratory). Tegaderm allowed us to collect up to 3 times more volume than with disposable diapers alone. These mock urine samples were analysed for phthalates and phthalate metabolites at a certified clinical laboratory and results showed that levels of phthalates and metabolites in all of these samples (with and without

tegaderm) were below the level of detection, suggesting minimal contamination from the tegaderm.

1 year visit:

Innate immune plates:

To permit longitudinal analysis, blood for innate immune responses was obtained and prepared for those 1 year old infants who had had plates prepared from their cord blood for innate immune analysis. At the 1 year time point, 444 subjects had a 6 hour incubation for intracellular cytokine analysis, 546 subjects had a 24 hour incubation for cell pellet analysis and 545 subjects had a 24 hour incubation for supernatant analysis. This protocol was identical to that used to assess cord blood exposing diluted whole blood to TLR ligands in a 96 well plate.

Saliva/buccal swab:

For children, the Oragene kit (OG-575, DNA Genotek, Kanata, ON)) was utilized. Saliva was collected using the provided sponges by rubbing inside the cheek until enough saliva was collected to reach the “fill line” of the collection container (approximate final volume 1.5 mL). The sample was aliquoted into two cryovials (0.75 mL each). For parents, the Oragene kit OG-500 was used. Donors were asked to spit into the collection tube until saliva reached the “fill line”. Two aliquots of 1.5 mL were placed in cryovials and then frozen at -80°C. Thus far, 23 maternal, 53 paternal and 157 one year child salivary samples or buccal swabs have been collected.

Storage, Logistics and Quality Control:

Health Diary:

Health Diary is designed to manage participant scheduling, case report forms, patient reported outcomes and to tabulate data in an instantly analyzable format compatible with SAS and SPSS analytical software. It conforms to the highest regulatory requirements, such as the Health Insurance Portability and Accountability Act (HIPPA) and the FDA Code of Federal Regulations 21 Part 1. The software is based on the Microsoft .NET platform and uses an SQL (Structured Query Language) server housed in a tier 3+ data centre with 99.99% guaranteed uptime and full 24/7 on-site security. Access to data is role based, SSL (Secure Sockets Layer) Encrypted and automatically copied to a secondary site. This web-based software platform was utilized to assign codes to samples, facilitate 2D bar code label printing (Brady printer, IP 300 Thermal Transfer Printer), track samples and print shipping manifests. The HealthDiary website also holds study protocols, tracks progress of sample processing and thus provides a central resource and reference for laboratory and study personnel.

Figure S1: Illustration of hognose roller dust collection tool

Rendered illustration of hognose roller vacuum dust collection tool exploded (left) and assembled (right). The body of the device was fabricated in aluminum, and the wheels and connectors in teflon to permit heat-sterilization and depyrogenation between collection sites and prevent marring of hard flooring surfaces. When assembled, each of the two barrels in the central tube of the collector houses a nylon dust collection thimble (DUSTSTREAM, Indoor Biotechnologies, Charlottesville, VA). The "double-barreled" collector provides a 2-fold increase in filter collection area over the manufacturer's standard collector, reducing the influence of dust

loading on collector back-pressure. For collection, the device is affixed to the nozzle of a household vacuum cleaner.

References

1. Blimkie D, Fortuno ES, 3rd, Yan H, 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.

Appendix: CHILD Sample Request Form

Application:

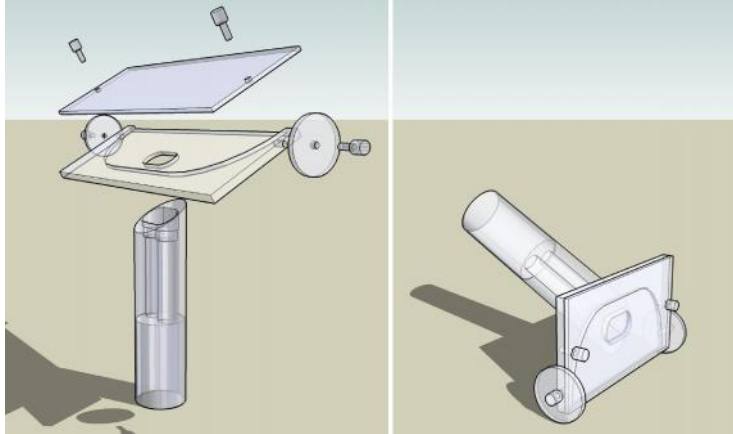
Principal Investigator(s):

Date:

REVIEW CRITERIA

1. Significance to CHILD goals: Briefly describe how the project advances knowledge about the genetic and environmental determinants of atopic diseases including asthma, allergy, allergic rhinitis, and eczema. Please elaborate how the proposed investigations link with or build on existing CHILD data or data to be collected in the CHILD study.
2. Investigator(s)/Environment: Please list the expertise, resources, infrastructure and funding available to support the proposed research.
3. Approach: What is the rationale for the methodology suggested? If applicable, please describe alternative approaches that were considered. Please describe any quality control measures in place for the proposed methodology.
4. Samples: Explicitly state and justify the number and type of CHILD samples required. Please describe steps taken to optimize the analysis to minimize the amount/volume of each sample needed. If the entire volume of a sample is not needed are their mechanisms in place to utilize the rest of the sample for alternate analyses? Is it possible to perform analysis on samples that have undergone freeze/thaw cycles?
5. Ethics/Review: Has this proposal received ethical review (locally or elsewhere)? Are any ethical concerns anticipated?

Figure S1: Illustration of hognose roller dust collection tool



Rendered illustration of hognose roller vacuum dust collection tool exploded (left) and assembled (right). The body of the device was fabricated in aluminum, and the wheels and connectors in teflon to permit heat-sterilization and depyrogenation between collection sites and prevent marring of hard flooring surfaces. When assembled, each of the two barrels in the central tube of the collector houses a nylon dust collection thimble (DUSTSTREAM, Indoor Biotechnologies, Charlottesville, VA). The "double-barreled" collector provides a 2-fold increase in filter collection area over the manufacturer's standard collector, reducing the influence of dust loading on collector back-pressure. For collection, the device is affixed to the nozzle of a household vacuum cleaner.

Table S1: Inclusion and exclusion criteria

Study subjects were required to meet the following inclusion and exclusion criteria:

Inclusion Criteria:

- [1] pregnant women aged 18 years and older (19 in Vancouver);
- [2] residence in reasonable proximity to the delivery hospital;
- [3] able to read, write, and speak English;
- [4] willing to provide informed consent;
- [5] willing to consent to cord blood collection for the study;
- [6] planning to give birth at a designated recruitment centre participating hospital;
- [7] infants born at or after 35 weeks;
- [8] able to provide name, address and telephone numbers of two alternate contact individuals.

Exclusion Criteria:

- [1] children born with major congenital abnormalities or respiratory distress syndrome (RDS);
- [2] expectation of moving away from a recruitment area within 1 year;
- [3] children of multiple births;
- [4] children resulting from in vitro fertilization;
- [5] children who will not spend at least 80% of nights in the index home.
- [6] children born before 35 weeks gestation

Table S2: Baseline demographics of cohort

General	Mean gestational age at enrollment (weeks)	26
	Mean maternal age (years)	32.3
	Siblings in home (% with 1 or more sibling)	48.3
Reported ethnicity	White Caucasian	2532 (72.9)
	SE Asian	428 (12.3)
	South Asian	91 (2.6)
	First Nations	177 (5.1)
	Black	77 (2.2)
	Other	161 (4.6)
	Unknown	6 (0.2)
Education	High school or less	295 (8.7)
	College or university	2443 (72.2)
	Postgraduate education	647 (19.1)
	Unknown	1 (0)
Health status	Reported asthma	760/3424 (22.2)
	Any reported allergy	2832/3425 (82.7)
	Atopic by skin testing	1768/3073 (57.5)
	Gestational diabetes	115/3475 (3.3)

Data are presented as n (%), except where there are varying numbers of missing data, when data are presented as n/N (%) where N is the total sample available for that variable.

Table S3: Aliquots (0.5 mL) of blood samples collected at prenatal visit

		Number of aliquots (%)						
		0	1	2	3	4	5	6
Maternal	Whole blood	9 (0.3)	6 (0.2)	3105 (99.5)				
	Serum	14 (0.4)	2 (0.1)	14 (0.4)	33 (1.1)	3057 (98)		
	Plasma	17 (0.5)	12 (0.4)	10 (0.3)	6 (0.2)	26 (0.8)	69 (2.2)	2980 (95.5)
Paternal	Whole blood	9 (0.4)	14 (0.6)	2319 (99)				
	Serum	18 (0.8)	5 (0.2)	12 (0.5)	39 (1.7)	2268 (96.8)		
	Plasma	17 (0.7)	12 (0.5)	15 (0.6)	33 (1.4)	116 (5)	236 (10.1)	1913 (81.7)

Table S4: Cord blood sample prioritization based on volume available (in centres not collecting for innate immunity assays)

Total volume heparinized blood collected (mL)	Whole Blood protocols				Post Ficoll protocols		
	2	3	4	5	6	7	8
		Total Volume - mL (number of aliquots)			Resuspension volume (mL)	Volume from Protocol 6 – mL (number of aliquots)	
6	n/a	0.5 (1)	0.5 (1)	1 (2)	5	1 (1)	4 (2)
10	n/a	1.5 (3)	0.5 (1)	2 (4)	8	2 (2)	6 (3)
12	n/a	2 (4)	1 (2)	2 (4)	9	3 (3)	6 (3)
15	n/a	2 (4)	1 (2)	3 (6)	12	4 (4)	8 (4)
17	n/a	2 (4)	1 (2)	3.5 (7)	14	4 (4)	10 (5)
20	n/a	2 (4)	1 (2)	4 (8)	17	5 (5)	12 (6)
23	n/a	2 (4)	1 (2)	4 (8)	20	6 (6)	14 (7)
25	n/a	2 (4)	1 (2)	4 (8)	22	6 (6)	16 (8)
27	n/a	2 (4)	1 (2)	4 (8)	24	6 (6)	18 (9)
31	n/a	2 (4)	1 (2)	4 (8)	26	6 (6)	>20 (10)
>31	n/a	2 (4)	1 (2)	4 (8)	>25	6 (6)	>20 (10)

Table S5: Cord blood sample prioritization based on volume available (in centres collecting for innate immunity assays)

Total volume heparinized blood collected (mL)	Whole Blood protocols				Post Ficoll protocols		
	2	3	4	5	6	7	8
		Total Volume - mL (number of aliquots)			Resuspension volume (mL)	Volume from Protocol 6 – mL (number of aliquots)	
6	2	0.5 (1)	0.5 (1)	0.5 (2)	3	1 (1)	2 (1)
10	2	1.5 (3)	0.5 (1)	2 (4)	6	2 (2)	4 (2)
12	2	2 (4)	1 (2)	2 (4)	7	3 (3)	5 (2)
15	2	2 (4)	1 (2)	3 (6)	10	4 (4)	6 (3)
17	2	2 (4)	1 (2)	3.5 (7)	12	4 (4)	8 (4)
20	2	2 (4)	1 (2)	4 (8)	15	5 (5)	10 (5)
23	2	2 (4)	1 (2)	4 (8)	28	6 (6)	12 (6)
25	2	2 (4)	1 (2)	4 (8)	20	6 (6)	14 (7)
27	2	2 (4)	1 (2)	4 (8)	22	6 (6)	16 (8)
31	2	2 (4)	1 (2)	4 (8)	26	6 (6)	20 (10)
>31	2	2 (4)	1 (2)	4 (8)	>25	6 (6)	>20 (10)

Table S6: Aliquots collected from 1 year maternal blood sample

	Number of aliquots						
	0	1	2	3	4	5	6
Whole blood	19 (0.7%)	12 (0.4%)	2768 (98.9%)				
Serum	12 (0.4%)	5 (0.2%)	11 (0.4%)	12 (0.4%)	2759 (98.6%)		
Plasma	25 (0.9%)	10 (0.4%)	5 (0.2%)	17 (0.6%)	42 (1.5%)	91 (3.3%)	2609 (93.2%)