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Version Post-print/Accepted Manuscript


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Kingston Allergy Birth Cohort (KABC); Exposome Characteristics and Parentally-Reported Respiratory Outcomes to Age 2

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Key Words:
Allergy, asthma, exposome, smoking, prenatal, epigenetics, mold, environmental health,
gestational age, breastfeeding, socioeconomic status, rural health, scented products

Abbreviations/Acronyms:
CI; 95% confidence interval, DOHaD; developmental origins of health and disease, FEV1;
forced expiratory volume in 1 second, HR; hazard ratio, KABC; Kingston allergy birth cohort,
KGH; Kingston general hospital, LICO; Canadian Low-Income Cut-Off, MNC; mononuclear
cells, NCD; non-communicable disease, NHS; national household survey, SES; socioeconomic
status.

Declaration of Sources of Funding
MLN; J.A. Stewart Award, Department of Medicine, Queen’s University, Ontario Ministry of
Research & Innovation Award, and AllerGen N.C.E. Canadian Allergy and Immune Diseases
Advanced Training Initiative (CAIDATI). The study was supported by an Ontario Thoracic
Society & Canadian Lung Association Grant-in aid of Research, an Allergy/Asthma Information
Association (AAIA) and Canadian Allergy, Asthma & Immunology Foundation (CAAIF) Award
for Ontario Research in Food Allergy, and an American College of Allergy, Asthma &
Immunology (ACAAI) Young Faculty Support Award.
Disclosures/Conflicts of Interest: The authors declare no conflicts related to this manuscript.

ABSTRACT WORD COUNT: 249
MANUSCRIPT WORD COUNT: max 4000
TABLES: 3
FIGURES: 3
Abstract

Background: The Kingston Allergy Birth Cohort (KABC) is a prenatally-recruited cohort initiated to study the developmental origins of allergic disease. Kingston General Hospital was chosen for recruitment, as it serves a population with notable diversity in exposures relevant to the emerging concept of the exposome.

Objective: Establish a profile of the KABC cohort using the exposome framework, and examine parentally-reported respiratory symptoms to age 2.

Methods: Data on phase 1 of the cohort (n=560 deliveries) were compiled and multivariate Cox proportional hazard models were used to determine associations with respiratory symptoms.

Results: The KABC exhibits diversity within the three exposome domains; “general external” (SES, rural/urban residency), “specific external” (cigarette smoke, breastfeeding, mold/dampness), and “internal” (respiratory health, gestational age). We demonstrate relationships between exposome domains, as residential locale and SES significantly affected characteristics of the home environment. Significant associations emerged between parental reports of wheeze/cough without a cold and exposures within all three domains, including SES, breastfeeding, gestational age, prenatal cigarette smoke exposure, and mold/dampness.

Conclusions: The KABC is a unique cohort with diversity that can be leveraged for exposomics-based studies, and a notably high prevalence of prenatal smoke exposure. This study demonstrated the impacts of all three domains of the exposome on the respiratory health of KABC children. Ongoing studies using phase 1 of the KABC are further exploring the internal exposome through allergy skin testing and epigenetic analyses, and the specific external domain through in-home environmental analyses, air pollution modeling and ultimately the potential convergence of the two.
Introduction

Developmental Origins of Allergic Disease and the Exposome

Allergic diseases are common in childhood, and although many risk factors are known, the increase in prevalence over the past quarter century remains poorly understood, and the probability of a substantial environmental contribution has been recognised \(^1\)\(^-\)\(^5\). Progress in research on the developmental origins of health and disease (DOHaD), also known as the Barker Hypothesis, has revealed \textit{in utero} and early-life effects on susceptibility to a wide range of adult diseases, such that it is now generally accepted that many non-communicable diseases, including allergic disease, are likely to arise from cumulative gene-environment interactions \(^6\), \(^7\). The exposome is a new conceptual framework that has been defined to encompass the totality of exposures encountered by an individual throughout the life-course, including the prenatal period \(^8\), \(^9\). The exposome framework requires consideration of both the nature of exposures, classified broadly into exposome domains; internal, specific external and general external, as well as their changes and cumulative impact over time \(^8\). The internal domain encompasses host factors, such as sex, age, metabolism, microbiome, epigenetics and inflammatory processes/diseases \(^8\). Specific external exposures are individual-level exposures, such as cigarette smoke and air pollution exposure, while general external exposures encompass wider social, economic and geographic factors, such as socioeconomic status (SES) and urban/rural residence, which also have direct effects on the individual \(^8\). Examining the exposome over time necessitates that birth cohort studies play a key role in exploring this new concept and applying it to gain a deeper understanding of the causes of chronic diseases \(^9\), \(^10\).

Studying Children with Varied Exposomes to Identify Modifiable Risk Factors
The Kingston Allergy Birth Cohort (KABC) is the first prospective study of allergic disease to be undertaken utilizing the unique catchment area served by Kingston General Hospital (KGH). By offering access to this unique population, with exposome variants that are not as prevalent elsewhere, it compliments larger Canadian cohort efforts such as the Canadian Healthy Infant Longitudinal Development (CHILD) Study. As an alternative to recruitment from specialized tertiary or quaternary hospitals, KGH offers access to a community that includes the City of Kingston (a small Canadian city with an approximate population of 152,000), and an approximately equal population residing in surrounding rural areas. In addition to coverage of the urban/rural divide in the province of Ontario, the KGH catchment area includes notable diversity in SES. Some Kingston neighborhoods are comprised of households that earn well above the Ontario provincial average, while others are archetypes of multi-generational poverty. Environmental and prenatal health issues, intimately related to SES, appear to disproportionately affect Kingston, such as prenatal smoking, which at 21.2%, is significantly higher than the rate in Ontario (12.4%), and the nearby city of Ottawa (7.7%). Thus, we chose KGH as the recruitment site in an effort to establish a unique cohort that encompasses exposome variants such as prenatal smoke exposure, while also including urban/rural participants and lower-SES families, in which the prenatal and early life risk factors of allergic disease can be explored.

The Kingston Allergy Birth Cohort Study Design

Recruitment for phase 1 of the KABC is complete and follow-up activities are either currently completed or underway as depicted in Figure 1. Briefly, survey data on environment and health
was collected during the third trimester, at 6 months, 1 year and 2 years, and surveys continue to be administered yearly as the children age. Allergy skin testing is being performed on mothers and children. Umbilical cord blood was collected from all available deliveries, and biological samples in the form of blood and plasma are being collected from the children at home visits, concurrent with indoor environmental sampling, and collection of blood and urine from the mothers. An outdoor air pollution sampling campaign was conducted in November/December 2014 for the measurement of NO₂ and NO for the development of the first land-use regression model of Kingston. Analyses of epigenetic biomarkers in relation to aspects of the prenatal and early life exposome are ongoing.

**Exposomic Analyses of Environmental Exposures and Health Outcomes**

The purpose of this paper is to characterize prenatal and early-life environmental factors in the KABC through the lens of the exposome, and assess potential associations with parental reports of respiratory symptoms to age 2. Herein we report that both urban/rural residence and SES were associated with prenatal and postnatal environmental exposures. Respiratory symptoms (parental reports of wheeze or cough without a cold) were significantly associated with prenatal cigarette smoke exposure, lower SES and the presence of mold in the home during early life. Increasing gestational age at birth, and breastfeeding for 6 months or more were negatively associated with early-life respiratory symptoms.

**Methods**

**Recruitment and Umbilical Cord Blood Collection**
Participants had to be able to communicate in English, and provide written informed consent prior to any study-specific procedures. Ethical clearance for the study was provided by the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board. Potential participants of the KABC were pregnant women, aged 18 years or older, in the second or third trimester. Participants were eligible if they intended to deliver at KGH, did not have pre-eclampsia or insulin-dependent diabetes, intended to retain custody of the child, were not intending to bank their umbilical cord blood, and did not exhibit any other risk factors that their physician felt would preclude their safe participation. Multiple births and consecutive siblings were not excluded. Participants were recruited via posters at family physician’s offices, midwives clinics, obstetrics clinics and at KGH. Potential participants were also invited to participate at clinic appointments, prior to scheduled Cesarian sections, or in early labor, if they responded positively to a query from a healthcare provider regarding their willingness to speak with study staff. Umbilical cord blood was collected in heparinized syringes at delivery. Cell types of interest were isolated fresh whenever possible and excess cord blood was processed to mononuclear cells for archiving in liquid N₂ in freezing medium. Umbilical cord blood was not collected from approximately 26% of women who consented to the study (Figure 1) due to failure to identify the woman as a study participant, cord blood loss from tearing of the cord or placenta or other factors surrounding the delivery.

Prenatal and Postnatal Surveys

A prenatal survey was administered at enrolment. The first 51 participants to enroll in the study were administered a less-detailed pilot survey. The pilot survey included questions regarding parental allergic disease, and maternal exposure to cigarette smoke. The full survey was
administered to all women who enrolled subsequently, and included additional questions regarding the home and other environmental exposures. Efforts were made to harmonize the information gathered in the full survey with the CHILD study, by adapting questions from Takaro and colleagues \(^1\), while limiting survey length/burden on the participants as much as possible.

Follow-up postnatal data collection is continuing, and the analyses presented herein focus on surveys administered at 6 months, 1 year, and 2 years following delivery, by mail or in person. If a child moved during the first 2 years of life it was determined whether or not the move changed the child’s classification in terms of any variables, and if so, the child was included under the classification where they spent 60% or more of their time under the age of 2. If that criteria was not able to be met, the status of the child with respect to that exposure was considered missing.

Definitions of prenatal and postnatal variables are given in Table 1.

**SES Estimation Using National Household Survey (NHS) Data**

Addresses were used to assign National Household Survey (NHS) \(^17\) data to each participant, by census-tract. If tract-level data was not available or censored for the neighborhood, the closest adjacent tract was used if the distance was less than 2 kilometers. If this was not possible, such as in rural areas, data for the rural town of residence, or census division were used.

**Definition of Early Life Respiratory Symptoms**
When reporting respiratory symptoms, parents were asked to only include wheezing and
coughing not associated with a cold. If they reported either respiratory symptom they were asked
how old the child was (in months) when the coughing/wheezing started.

**Statistical Methods**

Percentages of the cohort were calculated based on all non-missing for that category. We aimed
to examine the correlation structure of the exposome of KABC children during the *in utero*
period and early life, using the variables in Table 1. Thus, we computed “exposome globes”
separately for each of those developmental time periods. The exposome globe is a technique
similar to that of the “relevance network” to find correlations between expressed genes. Patel
and Manrai recently developed the relevance network technique into the exposome globe to aid
in visualizing and understanding relationships between various exposures in exposomics. We
computed the non-parametric correlation coefficients and p values between each pair of
environmental factors in R and generated exposome globes using Circos to visualize the top
quartile of correlation coefficients. P values were adjusted post-hoc using the Benjamini-
Hochberg false discovery rate for multiple tests of significance in R.

Correlations between exposures were used to inform our subsequent examination of factors
associated with parental reports of wheeze/cough without a cold. We employed a stepwise
analysis; we first explored the potential association between each exposure and respiratory
outcomes using univariate Cox proportional hazard models. All variables that exhibited p < 0.15
upon univariate analysis plus any variables they were significantly associated with in the
previous exposome globe analyses (Supplemental Tables 1 & 2), were entered into a
multivariate Cox proportional hazard regression model. The large number of significant relationships between the variables resulted in the majority of variables being included, thus the inclusion of variables was further optimized using Akaike information criterion (AIC). Prism version 6.0 (Graphpad Software Inc.) was used for graphing Kaplan–Meier curves and associated Fisher’s exact/Chi squared tests, while R was used for all other analyses R. Two-tailed p values < 0.05 were considered significant.

Results

Characteristics of the KABC Cohort

First, we set out to understand the basic exposome characteristics of the KABC participants at enrolment by collating survey data (Table 1). The general external exposures of residential setting and SES demonstrated diversity, as residential setting was almost evenly split between urban and rural, and incomes ranged from below the Canadian Low-Income Cut-Off (LICO), to well above the provincial average. More than a quarter of children were exposed to cigarette smoke in utero, and prenatal smoke exposure was an everyday occurrence for more than 60% of the children whose mothers reported being exposed to smoke (Table 1). The diversity in residential exposures reported in the KABC was also revealed the the broad range of potential pollution sources; the most common potential source within 100 meters of the home was a major road, while the second most common was a farm. Potential exposures within the home also varied, as more than two thirds of the cohort reported furry pets living in the home, one fifth reported mold or dampness in the home, and approximately half reported using air fresheners at least once a week (Table 1).
Follow-up Survey Responders Recapitulate the Diversity of the Cohort as a Whole

During the first 2 years of the study, 3% of women withdrew and 16% moved or otherwise became unreachable by phone, post and email (Figure 1). Of the 452 mothers remaining, 52% responded to at least one survey during the first 2 years of follow-up. The remaining 48% were non-responders for the purposes of this analysis (age 2 and under) but were not lost to follow-up, as they may contribute data to future analyses. Thus the prenatal survey analyses includes data from 560 children, while the postnatal includes 235. There were no significant differences between the whole cohort and those that responded to surveys in terms of urban/rural residence, SES, or proportions of mothers who self-reported having allergic disease (p>0.05, Fisher’s Exact test).

Correlation Structure of Prenatal and Postnatal Exposures

Characteristics of the home environment were assessed as part of the prenatal survey, and we observed strong evidence of interconnectedness between several individual factors (Figure 2A). SES, housing type, garage type and urban/rural residence were the factors investigated that were most highly linked to other aspects of the prenatal environment, with 8, 12, 9 and 7 significant associations with other exposures, respectively (adjusted p<0.05, Supplemental Table 1). Prenatal smoke exposure was significantly associated with housing type, home age, and the regular use of candles and air fresheners in the home (adjusted p<0.05, Supplemental Table 1). The presence of a dog in the home was associated with housing type and rural residence, while the presence of a cat was not significantly associated with any other home environmental factors (adjusted p<0.05, Supplemental Table 1). In addition to more indoor dogs, rural residence was
also significantly associated with a greater proportion of single family homes, wood heating, living near a farm, and higher SES, while it was negatively associated with living near a major road or parking lot (adjusted p<0.05, **Supplemental Table 1**). While the top 25% of correlation coefficients demonstrated connectedness of maternal and paternal allergy to other factors, they were not significantly associated with any other prenatal characteristics (**Supplemental Table 1**).

The interconnectedness of the “web” of environmental factors surrounding each child were also evident among the postnatal characteristics examined (**Figure 2B**). Similar to the prenatal time period, the postnatal factors that correlated significantly with the largest numbers of other factors included housing type, urban/rural status, and SES (**Supplemental Table 2**). Additional relationships emerged, such as significant associations between Cesarean section delivery, the presence of older siblings in the home and gestational age (adjusted p<0.05, **Supplemental Table 2**). Breastfeeding was associated with significantly lower rates of air freshener and candle/incense use (adjusted p<0.05, **Supplemental Table 2**). Home age was also significantly related to SES and newer homes contained significantly less carpeting (adjusted p<0.05, **Supplemental Table 2**). The exposome globe analysis and correlation analyses also revealed that KABC children growing up in lower SES homes were also significantly more likely to be exposed to mold in the home (adjusted p<0.05, **Supplemental Table 2**).

**Prenatal Factors Associated with Rates of Parentally-Reported Respiratory Symptoms**

Examination of correlation structures in exposome studies is meant to promote transparency and facilitate the interpretation of exposure effects in light of their relationships to other exposures.
Thus, we considered the interrelatedness of exposures as we went on to examine factors associated with parental reports of wheeze/cough without a cold. In our step-wise analysis, the prenatal presence of a cat in the home, the regular use of air fresheners, SES, prenatal smoke exposure, home age and garage type met the a priori criteria for inclusion in the multivariate model. These factors plus all variables that demonstrated significant associations with them in the exposome globe analysis were included in an AIC analysis for selection of the optimal model while minimizing loss of information. In the final multivariate model, adjusting for SES, cat in the home, gas heat, the use of air fresheners, and carpeting, only prenatal smoke exposure was significantly associated with parental reports of cough or wheeze without a cold to age 2 (adjusted p = 0.00006, Table 2). The overall incidence rate of parental reports of respiratory symptoms was 0.19 cases/person-year.

**Postnatal Factors Associated with Rates of Parentally-Reported Respiratory Symptoms**

In univariate Cox proportional hazard models of postnatal variables, gestational age, SES, indoor cat exposure, breastfeeding for 6 months or more, mold in the home, postnatal smoke exposure, and the regular use of air fresheners and candles met the criteria for potential inclusion in the multivariate model. All postnatal characteristics that significantly correlated with these variables of interest in the exposome globe analysis were also included (Supplemental Table 2). After AIC optimization of the model, increased gestational age at birth, the presence of siblings in the home and breastfeeding for at least 6 months were found to be significantly negatively associated with respiratory symptoms (adjusted p < 0.05, Table 3). The frequent use of air fresheners in the home (solid, plug-in or spray at least once a week) and the presence of mold in the home were
significantly associated with increased parental reports of wheeze or cough without a cold during the first 2 years of life (P<0.05, Table 3). Model effect estimates and p values for each variable of interest were adjusted for all other variables included in the multivariate model, including gestational age, breastfeeding, siblings, air fresheners and mold.

As the internal exposome characteristic of increased gestational age at birth was significantly associated with lower risk of respiratory symptoms, we further sought to determine if this effect was driven by children born prematurely. We excluded the 13 children who were born at ≤37 weeks gestation and found that the association remained significant in both univariate (hazard ratio = 0.74, p = 0.022) and multivariate models (hazard ratio = 0.75, adjusted p = 0.029). Thus, increasing gestational age is significantly associated with lower rates of parental reports of respiratory symptoms before the age of 2 in the KABC, across all births, as well as in term babies only.

**Prenatal and Postnatal Factors Identified Demonstrate Effects on Symptom Development on Kaplan–Meier Curve Analyses**

We visualized the effects of the variables found to have significant effects on respiratory symptom development using Kaplan–Meier curves (Figure 3). The curve of symptom development for prenatal smoke-exposed children was significantly different from unexposed, by the Log-rank Mantel-Cox test (p = 0.008), and the exposed/unexposed curves significantly departed from one another at 3 months of age (p = 0.008, Fisher’s exact test). The Kaplan–Meier curves of air freshener and mold exposure were also found to be significantly different from the
curves of their respective unexposed groups (p = 0.0065 and p = 0.01, respectively, by the Log-rank Mantel-Cox test). The symptom-development curve of children exposed to the regular use of air fresheners departed significantly from control at 12 months (p = 0.009, Fisher’s exact test), while mold-exposed children departed from control at 3 months (p = 0.006, Fisher’s exact test). Factors found to be protective against symptom development also demonstrated significant effects in Kaplan–Meier analyses (p = 0.02 for gestational age and p = 0.001 for breastfeeding), and departure from controls at 3 months (p = 0.04), and 9 months (p = 0.02). Kaplan–Meier analysis of the effect of siblings did not demonstrate significance.
Using the Exposome Framework in Non-Communicable Disease Research

The purpose of the exposome concept is to improve identification of environmental risk factors in epidemiological studies and potentially gain new insight into critical windows of vulnerability throughout the life course. The examination of the pregnancy exposome and the development of the exposome globe concept have begun to provide early insights, and new techniques for data analysis in exposomics are ardently being developed and tested. Large consortia studies, such as the human early-life exposome (HELIX) collaboration of 6 European cohorts are using “big health data” and new techniques such as smartphone applications to take important steps towards measuring the life-course exposome. However, there are also significant challenges in applying the exposome concept and subsequently acting upon these findings to reduce individual or population risk. Here we have attempted to align environmental exposures within a new birth cohort with this concept to begin to turn it into practice by examining childhood cough and wheeze for the first time through the lens of the exposome. We hypothesize that starting with birth cohorts, such as the KABC, provides the greatest opportunity to fully embrace and test the concept, yet considerable challenges can be expected as the exposome approach evolves.

Non-communicable diseases (NCDs) have been declared to be a worldwide public health crisis, and allergic diseases, including asthma, are the most common NCDs of childhood. In this study we analyze longitudinal survey data from the third trimester to 2 years of age using the exposome globe technique, and we examine parentally-reported respiratory health outcomes. In ongoing follow-up studies we are making more in-depth measures of the specific external
exposome, including environmental sampling in the bedrooms of the children, and measures of
the internal exposome, such as genome-wide epigenetic biomarkers in umbilical cord blood and
peripheral blood. Using the exposome framework may lead to a better understanding of
modifiable risk factors as well as the basic mechanisms underlying the effects of these
exposures, individually and collectively.

**Links Between the Three Domains of the Exposome**

Using the KABC cohort, we examined relationships between the three domains of the exposome;
internal, specific external, and general external. We found that the general external domain
factors of SES and urban/rural status were strong modifiers of the specific external exposome, as
they were significantly associated with multiple exposures such as housing type, age of the
home, indoor dog ownership, wood heating, proximity to traffic pollution and even consumer
product behaviors such as the use of air fresheners in the home.

The use of the exposome globe concept brought to light some interesting relationships that are
sensible, but may not have been recognized otherwise, such as the associations between
Cesarean section, the presence of older siblings in the home and gestational age. This is likely
due to repeat elective Cesarean sections following the previous Cesarean delivery of an older
child, and the scheduling of the surgery in advance of the due date. This is a fascinating
example of how an individuals’ exposome extends to the pre-conception stage, as the mother’s
previous pregnancy history is linked to the subsequent child’s mode of delivery and gestational
age; factors that have the potential to change their disease risk across their life course.
In the analysis of parentally-reported respiratory symptoms, the importance of links between different exposures and exposome domains remained evident. Inclusion of other factors known to be associated with the predictors of interest improved our ability to ensure we were controlling for as many potential confounders as possible. Thus, the exposome concept helped to organize our thinking around risk factors in the KABC cohort; how they are related and influence each other. As follow-up continues and biological samples are analyzed, the exposome concept may aid in identifying novel risk factors, biomarkers of exposures, or exposure-classes.

**Gestational Age and Parental Reports of Wheeze/Cough in Early Life**

As a host factor that we carry with us for our entire lives, gestational age at birth is an important aspect of the internal exposome. It is known that preterm birth is the leading cause of child death in high-income and middle-income countries, and the USA is one of the ten countries with the highest numbers of preterm births \(^{29}\). The percentage of children in the KABC who were born preterm was consistent with the overall 4.9% rate of preterm birth in Canada \(^{30}\). However, the negative association between weeks of gestation and reports of respiratory symptoms in the KABC was not limited to preterm deliveries. Many follow-up studies of lung function focus on children born very preterm (<32 weeks), and demonstrate persistent negative effects \(^{31}\). Recently, children in the Children Allergy Milieu Stockholm Epidemiological Survey (BAMSE) cohort born moderate-to-late preterm were examined, revealing that these groups also experience significant decrements in forced expiratory volume in 1 second (FEV1) \(^{32}\). Relatively little research has focused on the effects of gestational age on respiratory health in term infants, but one study recently demonstrated an increased risk of asthma in early-term births (37-38 weeks, adjusted odds ratio,
1.2; 95% CI, 1.1-1.4), and a decreased risk of asthma in those born at 41 weeks or later (adjusted odds ratio, 0.9; 95% CI, 0.8-1.0) 33. This is consistent with our findings, suggesting that increases in gestational age across the entire spectrum may be beneficial in terms of reduced risk of respiratory disease.

Maternal Smoking During Pregnancy

Maternal smoking is a crucial modifiable prenatal risk factor that is known to profoundly affect the health of children. A recent meta-analysis of prospective cohorts determined that prenatal smoke exposure was significantly associated with incident asthma 34. While we are not able to determine incident asthma from the parental-report data that is the focus of this analysis, the significantly higher rate of wheeze/cough without a cold in the KABC children who were exposed prenatally is consistent with the previous meta-analysis of Burke et al.

While we focused on one area known to be affected by high maternal smoking rates, there are many other pockets of high-risk populations across North America. Geographically, clusters of higher than average maternal smoking rates have been shown to exist in the American Midwest 35, and Western Canada 36. Other factors such as economic-vulnerability 35, 37, education 38, maternal age less than 24 35, 36, 38, cultural background 39, and experiencing stressful events before/during pregnancy 40 are all associated with increased risk of maternal smoking during pregnancy. While smoking rates in the United States have fallen over the past 50 years, during the past 10 years tobacco-control efforts have been insufficient to reach national objectives of reducing prenatal smoking 38, 41. Thus, maternal smoking during pregnancy remains a pressing public health issue. A spatiotemporal external exposome framework has previously been used to
examine “hotspots” where the prevalence of low birth weight was higher than expected in the United States \(^{42}\). While Kingston was a known “hotspot” for maternal smoking that was targeted for this study, applying a longitudinal spatial exposome analysis applied to maternal smoking may provide new knowledge for targeted interventions in the future.

**Breastfeeding and Siblings**

In this study, both breastfeeding and the presence of siblings in the home were associated with a lower incidence of parentally reported respiratory symptoms. Breastfeeding during early life is an important aspect of the specific external exposome, which may have long-term effects on the internal exposome, such as aiding in the establishment a healthy gut microbiome, as well as epigenetic and metabolomic effects\(^{11,43-45}\). Siblings have also been shown to affect gut microbiota development in early life and previously related to rates of asthma and allergies \(^{46,47}\).

The rate of breastfeeding reported herein is comparable to the rate of 53.1% determined for non-exclusive breastfeeding to at least 6 months by the Canadian Community Health Survey \(^{48}\). The relationship between breastfeeding and the risk of childhood asthma has been studied extensively, but remains controversial. A number of studies have demonstrated protection, while others report no effect, or even increased risk among breastfed children, particularly if the mother is atopic \(^{49-51}\). In the KABC we found a negative association between breastfeeding for at least 6 months and reports of wheeze or cough without a cold. There was no difference between allergic and non-allergic KABC mothers in terms of breastfeeding rates, or reports of respiratory symptoms in their children. Thus in this cohort, breastfeeding appears to have positive effects towards respiratory health outcomes in the children of both atopic and non-atopic mothers.
Although some studies fail to find a specific effect of breastfeeding on rates of asthma, it is widely recognized that breastfeeding provides a range of benefits for growth, immunity, and development. The prospective data collected by the KABC on breastfeeding duration and solid food introduction will be useful for further studies on the developmental origins of respiratory health when skin testing, epigenetic and pulmonary function data become available on these children.

Indoor mold and scented product exposures

Airborne specific external exposures in the home, such as indoor mold and air fresheners may be particularly relevant to respiratory health in early life. In cross-sectional studies and meta-analyses, mold exposure has been linked to increased risk of allergic respiratory health outcomes. Beta-glucan, a polymer present in fungal cell walls, was demonstrated to be associated with persistent atopic asthma and bronchial hyper-responsiveness in another Canadian cohort. Our results are in line with these findings, as we demonstrate that the strongest postnatal association between increased reports of wheeze/cough and any variable was with mold/dampness in the home.

The use of scented products in the home is another emerging indoor air quality issue. A recent study demonstrated that in addition to perfume-related chemicals, scented products also contain high levels (>1000μg/g) of phthalates. Links have been made between exposure to phthalates and childhood asthma, however, further longitudinal studies are needed to determine causality. Phthalate levels will be measured in samples taken during home visits in the KABC cohort and...
correlations between health outcomes and epigenetic effects in blood samples collected concurrently may emerge.

**The Time-Integrated Exposome**

By definition, the exposome changes over time based on cumulative exposure. We found that different groups of variables behaved differently in terms of their level of continuity between the prenatal and postnatal periods examined. For example, urban/rural residence, housing type, and SES did not change unless a child moved into a new home that changed their classification in terms of those variables. Less than 9% of the children displayed discordance between those prenatal and postnatal variables. While other variables, such as mold in the home, the use of air fresheners and smoke exposure have the prospect of changing between the prenatal and postnatal periods without the family moving. For example, air fresheners and mold exposure each demonstrated discordance in approximately 30% of children from the prenatal to postnatal periods. Both of these exposures were found to be significantly associated with respiratory symptoms when the exposure occurred postnatally. This may point to biological mechanisms such as placental protection and/or a mechanism that is dependent on direct exposure of the child's lungs.

Smoking provided another example of the importance of the time-integrated exposome. While the total percent of the cohort exposed to cigarette smoke did not change dramatically from the prenatal (26%) to postnatal (23%) periods, the degree of smoke exposure of the children appeared to change significantly, from 16% of prenatal smoke being an everyday occurrence, to only 2% of children who were reported to be exposed to smoke in the home after birth, with the
remaining 21% representing third-hand or second-hand somewhere other than the home. In addition, the parents of 32 children (almost 14% of survey responders) who did not report any smoke exposure during pregnancy, did report postnatal smoke exposure, thus some children changed exposome characteristics dramatically. The more chronic and consistent nature of the prenatal smoke exposure, or the developmental timing, or both, may play roles in our finding that prenatal smoke exposure was significantly related to respiratory symptoms to age 2, while postnatal smoke was not. It is possible that with a larger sample size, or with additional data collection later in life, the effects of postnatal smoke exposure will become evident. Thus the effects of several exposures found to affect respiratory health in this study may be as a result of the time integrated pre-natal exposure or the time-integrated exposure during infancy, or both.

**Time to Onset of Symptoms**

The postnatal time point at which the Kaplan–Meier curves of the exposed children departed from the unexposed also revealed some intriguing differences between the exposome characteristics of interest. The effects of prenatal smoke exposure, gestational age and postnatal mold exposure were significant by 3 months of age. Both prenatal smoke exposure and gestational age have been associated with potentially-permanent changes in lung development. While for mold exposure one explanation might be immediate irritating effects upon inhalation of mold in infants, although the indoor microbial environment is complex and additional studies will be needed to further assess mechanisms. The Kaplan–Meier curves of children who were not breastfed for at least 6 months and those who were exposed to the regular use of air fresheners indoors significantly diverged from control at 9 months and 12 months, respectively.
The 9 month time point at which the effects of breastfeeding became apparent may be due to the time it takes for the establishment of the microbiome and other immune-modulating factors associated with breastfeeding. Air fresheners represent a complex mixture of chemicals with various toxicological profiles. Thus, the differences in the onset of symptoms of each group of children with the exposome characteristics of interest may reflect the differences in the impacts of their time integrated exposures.

Limitations of the Kingston Allergy Birth Cohort (KABC)

The generalizability of studies is known to depend heavily on both the population and the sampling scheme. Some of our recruitment was carried out through posters at family physician’s offices, midwives clinics, and KGH. It is possible that the participants who contacted us through this route were interested because someone in their family had been affected by allergic disease. However, ~90% of those in the study were approached in person at appointments, prior to scheduled Cesarian sections, or in early labor, and 79% of women who were asked to participate in the study consented. Thus, we acknowledge that there may be some participation bias, but conclude that it is not likely to have caused overwhelming deviations from the general population. However, as study staff regularly attended the labor and delivery ward prior to scheduled Cesarean sections to ask if women may be interested in the study, our study population does encompass a higher than average number of Cesarean section births (53% in the KABC compared to 25.9% in the public health region). The percentage of smoking mothers in the KABC is consistent with recent public health reports for the region, suggesting that the smoking population in Kingston was not underrepresented. Representation from both the highest and lowest SES census tracts also demonstrates that the cohort adequately captured the
notable diversity of SES of the Kingston area. While the percentage of eligible women in the cohort who completed follow-up surveys was lower than ideal, at just over 50%, the diversity in the sample in terms of prenatal smoking and SES remained. Some women who did not respond to surveys to age 2 have already provided data for the ongoing collection at age 3, and attempts at follow-up will continue.

In this study we did not find an association between self-reported proximity to traffic pollution and respiratory symptoms in the children. However, studies have found that self-reported air pollution exposure is only weakly associated with modeled air pollution exposure. In the CHILD study, postnatal exposure to traffic-related air pollution was associated with atopy at 1 year of age, using models that incorporated temporal variability and residential mobility. We are currently working to quantify measures of the specific external exposure of outdoor air pollution at the home address through the development of a traffic-related air pollution model for Kingston based upon a short-term campaign of NO₂ and NOₓ measurement. Particularly because of the inclusion of both urban and rural participants in the KABC, significant gradients in exposure may be present, and further studies may be able to exploit this pattern.

Another limitation to the study is that, save for SES, we did not collect measures of the prenatal and postnatal psychosocial environment and stress, which may be involved in child development and the prevalence of allergic and other diseases. The KABC was designed for recruitment to be carried out in phases, and plans are being made to collect more in-depth psychosocial health and stress exposure data in phase 2. Further phases will also increase the overall sample size and
provide the opportunity to validate the associations made herein, while delving deeper into potential biological mechanisms.

Conclusions

The present study introduces a new cohort with a high prevalence of in utero exposure to cigarette smoke, drawn from a mixed population of small-city urban and rural residents, with diverse socioeconomic status. This cohort has the advantage of exhibiting diversity within the three exposome domains. We demonstrated interplay between the general and specific exposome domains, as urban/rural status and SES was demonstrated to significantly affect characteristics of the home environment. This is the first to examine the development of cough and wheeze without a cold in early childhood through the lens of the exposome. We report increased incidence associated with prenatal cigarette smoke exposure, mold/dampness in the home and the use of air fresheners. Breastfeeding for 6 months or more and increasing gestational age were associated with decreased respiratory reports. These findings will be followed by further investigations of the home environment, and the internal exposome including epigenetics and metabolomics. We will continue to approach the cohort from the exposome perspective and attempt to advance that thinking further for KABC and the ‘exposome community’ in general.
Figure Legends

**Figure 1:** Flow chart of Kingston Allergy Birth Cohort (KABC) enrolment, withdrawal, loss to follow-up and follow-up survey completion.

**Figure 2:** Exposome globes depicting the relationships, in terms of non-parametric correlation coefficients, between components of the A) prenatal and B) postnatal environments.

**Figure 3:** Kaplan–Meier curves depicting the effects of A) prenatal smoke, B) the regular use of air fresheners in the home, C) postnatal mold exposure in the home, D) gestational age, E) breastfeeding for at least 6 months, and F) the presence of siblings in the home on parental reports of symptoms of wheeze and cough without a cold.

Acknowledgements

KABC Investigators also include Dr. Michael Kobor, Dr. Meaghan Jones. The study staff would like to thank the families who took part in the study and the doctors, midwives and nurses at Kingston General Hospital. Thank you to Mena Soliman, Jenny Thiele, Michelle Roddy, Roberta Faroldi, Kiyoka Sasaki, Sara Angélica Urquieta Ruelas, Katie Corscadden, Ibrahim Sandoqjy, Julianne Murphy, Nancy Rogers, Katrina Au, Kerolyn Shaursingh, Dr. Mohammad Alavinia, Yuchao Wan, Congqiao Yang and Professor Marianne Hatzopoulou for their assistance on various aspects of the KABC.


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