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ABSTRACT #1

The effects of infant feeding practices on food sensitization in a Canadian birth cohort

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BACKGROUND
Evidence regarding the impact of infant feeding practices, including breastfeeding and timing and diversity of food introduction, on atopic sensitization remains controversial. We examined the relationship between infant feeding and development of sensitization to foods at age 1 in the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort study.

METHODS
Nutrition questionnaire data prospectively collected at age 3, 6, 12, 18, and 24 months were used to characterize timing of introduction of cow's milk products (CMP), egg, and peanut/peanut butter; diversity of food introduction; and exclusive breastfeeding to 6 months. At 1 year, infants underwent skin prick testing to cow's milk, egg white, and peanut, with a wheal diameter ≥ 2 mm regarded as positive. To allow the ability to account for potential confounders, we analyzed data from children with complete data for timing of food introduction and skin prick testing for the infant and both parents (n=1421).

RESULTS
Most parents introduced CMP early (0-6 months 48%, 7-12 months 48%, ≥ 12 months 4%) but delayed introduction of egg (0-6 months 6%, 7-12 months 76%, ≥ 12 months 19%) and particularly peanut (0-6 months 1%, 7-12 months 41%, ≥ 12 months 58%). Exclusive breastfeeding to 6 months was low in our cohort (23%). At age 1 year, 10% of children were food-sensitized, with highest prevalence of egg white sensitization (6%). Early introduction of CMP, egg, and peanut was protective against sensitization to the corresponding food allergens. Introducing egg before age 1 significantly reduced the odds of developing sensitization to any of the three tested food allergens (0-6 months adjOR 0.33, 95% CI 0.12-0.90; 7-12 months adjOR 0.47, 95% CI 0.31-0.73), after adjusting for study center, parental atopy, and parental ethnicity. The benefits of early introduction of these foods in reducing the risk of sensitization were more evident in male children. Exclusive breastfeeding to 6 months did not affect the risk of sensitization to foods, except for cow's milk (adjOR 4.66, 95% CI 1.31-16.61). Within the three allergenic foods assessed, less diversity of introduction before age 1 was associated with greater risk of food sensitization.

CONCLUSIONS
Early introduction of solid foods reduced the risk of food sensitization, especially in male children, as did an increased diversity of these allergenic foods introduced during the first year. The findings from this study reaffirm the paradigm shift from delayed food introduction and food avoidance to earlier introduction of diverse foods for allergy prevention.

ABSTRACT #2

Sensitization and allergy to highly-allergenic foods at age 3 years

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BACKGROUND
We evaluated the associations between the development of sensitization and food allergy diagnoses for milk, egg and peanut at age 3 years, with the goal of contributing to our understanding of why only some food-sensitized children develop an allergy to the food.

METHODS
Caregivers of participants in the population-based Canadian Healthy Infant Longitudinal Development (CHILD) Study prospectively reported their child's introduction and reactions to foods in the first 3 years of life. At ages 1 and 3 years, the children underwent skin prick testing to foods including milk, egg and peanut and assessment by an allergist or trained researcher for food allergies and other allergic conditions. We evaluated the patterns of food sensitization and healthcare provider-diagnosed food allergies at age 3 years.

RESULTS
In preliminary data (n=2644), sensitization (skin prick test mean wheal diameter ≥ 2 mm) was 1.10% to cow milk, 2.31% to egg and 3.87% to peanut at age 3 years. Between ages 1 (n=2935) and 3 years, some children lost sensitization to milk (1.40%), egg (5.58%) and peanut (2.35%) and others were newly sensitized (0.74%, 0.78% and 1.37%, respectively). Diagnoses of allergy to milk, egg and peanut were made for 13.8%, 16.4% and 21.6% of children sensitized to each of these foods, respectively. Of children with a diagnosis of allergy to milk, egg and peanut, 40.0%, 58.8% and 84.6% were sensitized to the food to which they were allergic. Sensitized children had higher odds of being diagnosed with an allergy (70-fold increase for milk and egg, and 170-fold increase for peanut).

CONCLUSION
Most children with sensitization to milk, egg and peanut at age 3 years were not diagnosed with an allergy to these foods; however, sensitization was a strong predictor of allergy diagnosis. Future work will examine dietary and other predictors of food sensitization and allergy to further our understanding of how sensitization to a food becomes a food allergy and ultimately, to work towards the prevention of food allergy development.

ABSTRACT #3

Maintenance milk oral immunotherapy at 9 months is associated with ongoing increases in casein-specific serum IgG4

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BACKGROUND

Cow's milk allergy (CMA) affects 2-5% of children [1] and is a cause of severe allergic reactions and anaphylaxis in children. Desensitization through milk oral immunotherapy (OIT) is an expanding area of research, where the duration of OIT for induction of tolerance remains unclear [2]. We report on the first randomized controlled trial in Canada evaluating milk oral immunotherapy (OIT) in children. We compared changes in skin prick tests (SPT) between commercial milk extract, fresh milk, and 1:10 diluted fresh milk (fresh milk-1:10) during milk OIT. We also assessed casein-specific serum IgE, IgG4, and IgA in subjects who successfully completed milk OIT.

METHODS

We performed an interim analysis for the first 10 subjects who completed the escalation phase to 200ml and for the first 5 subjects who continued maintenance doses for 9 months post escalation phase (9M Post OIT). SPT to milk extract, fresh milk-1:10, and fresh milk were evaluated at baseline, 200 ml, and 9M Post OIT. Casein- specific serum IgE, IgG4, and IgA were evaluated at baseline, 200 ml, and 9 M Post OIT. Statistical analysis was done using the paired t-test.

RESULTS

There was a statistically significant decrease in SPT from baseline to 200 ml for milk extract (mean difference 4.36mm, 95% CI 0.78-7.95mm), fresh milk-1:10 (mean difference 5.55mm, 95% CI 1.62- 9.47mm), and fresh milk (mean difference 6.46mm, 95% CI 2.13-7.05mm). SPT did not significantly decrease at 9M Post OIT compared to 200ml. In parallel, casein-specific serum IgE decreased significantly from baseline to 200ml (mean difference 14.41ng/ml, 95% CI 1.70-21.11ng/ml) and did not significantly decrease at 9M Post OIT compared to 200ml. Similarly, casein-specific IgA decreased significantly from baseline to 200ml (mean difference 24.98ng/ml, 95% CI 0.71-49.26ng/ml), and did not significantly decrease at 9M Post OIT compared to 200ml. Serum IgG4 to casein increased significantly from baseline to 200ml (mean difference 1255ug/ml, 95% CI 28.75-2482ug/ml), from baseline to 9M Post OIT (mean difference 2582ug/ml, 95% CI 614.99-4550ug/ml), and from 200ml to 9M Post OIT (mean difference 1840ug/ml, 95% CI 233.02-3446ug/ml).

CONCLUSION

Successful escalation phase of milk OIT in IgE-mediated CMA in children is associated with decreases in milk-specific SPT and casein-specific serum IgE and IgA, and increases in casein-specific serum IgG4. There are further increases in IgG4 during maintenance milk OIT at 9 months post escalation phase. This suggests there are ongoing immunological changes during maintenance milk OIT and should be continued long-term.

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ABSTRACT #4

Increased drug-induced anaphylaxis visits and factors affecting reaction severity: a 3-year follow-up study in two Emergency Departments in Montreal

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BACKGROUND
While previous studies have suggested increased rates of anaphylaxis, it is not clear if the rate of drug-induced anaphylaxis is increasing [1, 2]. We aimed to assess the percentage of drug-induced anaphylaxis cases among all Emergency Department (ED) visits due to anaphylaxis in a pediatric and an adult ED in Montreal and to determine factors associated with severe reactions.

METHODS
Over a 3-year period, children and adults presenting to the Montreal Children's Hospital and Hôpital du Sacré-Coeur ED with anaphylaxis were recruited as part of the Cross-Canada Anaphylaxis REgistry (C-CARE). A standardized data entry form documenting symptoms and triggers of anaphylaxis was collected by the physician. Patients were contacted every 12 months to inquire if the anaphylaxis drug trigger was confirmed. Multivariate logistic regression was used to estimate factors associated with reaction severity.

RESULTS
From June 2012 to May 2015, 29 patients presented to the pediatric ED with drug-induced anaphylaxis of which 37.9% were male with a median age of 9.67 years (IQR: 4.6, 15.6). Of these reactions, 6.9% (95%CI, 0, 16.7) were classified as severe and 34.5% (95%CI, 16.1, 52.9) of patients reacted to antibiotics (Table 1). More than half the patients (58.6% [95%CI, 39.6, 77.7]) were treated with epinephrine. At the adult ED, 52 patients presented with anaphylaxis to drugs of which 25.0% were male with a median age of 49.1 years (IQR: 38.0, 62.9). 17.3% (95%CI, 6.7, 27.9) of the reactions were severe and 59.6% (95%CI, 45.8, 73.4) of patients reacted to antibiotics (Table 1). About half of the patients (48.1% [95%CI, 34.0, 62.1]) were treated with epinephrine. The percentage of drug-induced anaphylaxis cases at the Montreal Children's Hospital and Hôpital du Sacré-Coeur demonstrated a raw increase from Year 1 to 3 of 13.8% (95%CI, 0.77, 65.9) and 9.62% (95%CI, 2.56, 47.4), respectively.

At the pediatric ED, severe reactions to drugs were associated with parenteral contact route and hospital admittance (OR 1.41 [95%CI, 1.16, 1.72] and 1.41 [95%CI, 1.05, 1.89]). At the adult ED, severe reactions to antibiotics were associated with fluoroquinolones and hospital admittance (OR 1.29 [95%CI, 1.04, 1.58] and 1.49 [95%CI, 1.06, 2.09]).

CONCLUSION
Caregivers should be aware of the risk of severe reactions in adults treated with fluoroquinolones and those requiring IV treatment. Given the increased percentage of ED visits and that 50% of reactions in children and adults were not treated with epinephrine, educational programs prompting the use of epinephrine in drug-induced anaphylaxis are required.

Table 1. Characteristics of Patients Presenting to the Emergency Department with Drug-Induced Anaphylaxis

Variable (%, 95%CI)	Montreal Children's Hospital	Hôpital Sacré-Coeur
N	29	52
AAR (median, IQR)	9.67 (4.6, 15.6)	49.1 (38.0, 62.9)
Sex (% males)	37.9 (19.1, 56.7)	25.0 (12.8, 37.2)
Cases per year		
2012 – 2013	27.6 (10.3, 44.9)	28.8 (16.1, 41.6)
2013 – 2014	31.0 (13.1, 48.9)	32.7 (19.5, 45.9)
2014 – 2015	41.4 (22.3, 60.4)	38.5 (24.8, 52.1)
Medication type		
Antibiotics	34.5 (16.1, 52.9)	59.6 (45.8, 73.4)
Non-Antibiotic Drugs	65.5 (47.1, 83.9)	40.4 (26.6, 54.2)
Known Drug Allergy	6.9 (0, 16.7)	30.6 (17.2, 44.0)
Known Food Allergy	20.7 (5.0, 36.4)	12.2 (2.7, 21.8)
Known Asthma	24.1 (7.6, 40.7)	4.1 (0, 9.8)
Reaction type		
Mild[1]	13.8 (0.4, 27.1)	0
Moderate[2]	79.3 (63.6, 95.0)	82.7 (72.0, 93.3)
Severe[3]	6.9 (0, 16.7)	17.3 (6.7, 27.9)
Treatment in ED		
Epinephrine	58.6 (39.6, 77.7)	48.1 (34.0, 62.1)
Antihistamines	41.4 (22.3, 60.4)	78.8 (67.4, 90.3)
Steroids	17.2 (2.6, 31.9)	86.5 (76.9, 96.1)

[1]Symptoms include urticaria, erythema, angioedema, oral pruritus, nausea, nasal congestion, sneezing, rhinorrhea or throat tightness
[2]Symptoms include crampy abdominal pain, diarrhea, recurrent vomiting, dyspnea, stridor, cough, wheeze, or "light-headedness"
[3]Symptoms include cyanosis, hypoxia, respiratory arrest, hypotension, dysrhythmia, confusion, or loss of consciousness.

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ABSTRACT #5

Mothers of children with food allergy: a discourse analysis of mothering activities

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BACKGROUND
Up to 550 million people worldwide experience food allergy and the incidence is increasing annually [1]. Living with food allergy is more difficult than is generally appreciated [2]. Challenges associated with most areas of daily living have been documented for both people with food allergy and their families [3, 4, 5, 6]. The majority of people with food allergy are children and two studies have reported that mothers and fathers experience their child's food allergy differently and that food allergy may have a more significant impact on the well-being of mothers [3, 4]. While there is an abundance of literature related to mothering children with other chronic diseases such as atopic dermatitis, asthma, and/or juvenile diabetes, there are few relevant scientific enquiries into mothering a child with food allergy [7, 8, 9]. Food allergy may share some common features with other chronic diseases. However, despite these commonalities there is benefit to disease-specific approaches to understanding the issues of mothering [8]. The purpose of this study was to explore how mothers of children with food allergy use language to discursively represent their mothering activities and thereby their maternal identities in response to having a child with food allergy.

METHODS
Discourse analysis was used to analyze text of 3 on-line blogs (356 posts) written by mothers of children with food allergy. Data collection began with a Google search of "Canada food allergy mom blogs". A link was then followed to the first blog that appeared which was found to be consistent with study parameters. Two additional blogs were accessed using the search terms "Australia food allergy mom blogs" and "America food allergy mom blogs". The first blogs to appear were considered and subsequently chosen as they both met study parameters. Data analysis included descriptive/analytical coding followed by interrogation of data for emerging topic areas. Several criteria for ensuring quality were considered throughout this study.

RESULTS
Four distinct but interrelated representations of mothers' identity based on mothering activities emerged through analysis of data. They are: (a) mothers as change managers; (b) mothers as "allergy moms"; (c) mothers as warriors; and (d) mothers as advocates.

CONCLUSIONS
This study advances the scholarship of food allergy by providing the first known exploration of mothering activities and resultant maternal identities relating to mothering a child with food allergy.

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ABSTRACT #6

Distinct trajectories in depressive symptoms and perceived stress from pregnancy to the postnatal period

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BACKGROUND

Despite recent interest [1], little is known about the determinants of maternal stress and depression trajectories from pregnancy to the postnatal period in Canadian society. This study aimed to: (1) identify distinct trajectories of pre/postnatal maternal perceived stress and depressive symptoms, (2) assess longitudinal associations between these two maternal distress constructs and (3) test associations between maternal distress trajectories and sociodemographic characteristics, such as immigration status.

METHODS

The sample consists of 3307 women participating in the Canadian Healthy Infant Longitudinal Development (CHILD) Study. Data on depressive symptoms (using Center for Epidemiologic Studies Depression Scale) and perceived stress (using Perceived Stress Scale) were collected at recruitment (mean = 27 weeks) and at 36 weeks of gestation in the prenatal period, and then at six months, one year, 1.5 years, and two years in the postnatal period. Data on maternal demographics, immigration status, depressive history and employment were collected at recruitment. General growth mixture models were conducted in Mplus 7.3 to identify distinct trajectories in depressive symptoms and perceived stress. Configural frequency analysis examined the associations between trajectory memberships. Multinomial regression was used to test how trajectory group memberships were related to maternal sociodemographics, such as immigration status and employment.

RESULTS

Five trajectory patterns for depressive symptoms (*chronic*: 2.2%; *antepartum*: 5.6%; *postpartum*: 7.6%, *never-higher scores:* 27.7%, *never-low score*s: 56.9%) and five trajectory patterns for perceived stress (*chronic*: 6.9%; *antepartum*: 6.3%; *postpartum*: 22.6%, *never-higher scores*: 40.2%, *never-low scores*: 24%) were found. Chronic stress was significantly associated with chronic depression or depression in the antepartum or postpartum period. Women with elevated levels of depressive symptoms in the antepartum period were at significant risk of higher stress across pregnancy and the first 2 years after delivery. Also, women from the two never stressed groups were significantly unlikely to suffer from depression at any point of the studied period. New immigrant women who lived in Canada for 6 to 10 years, but not newcomers who moved to Canada within the last five years, were at a higher risk for chronic and postpartum depression.

CONCLUSIONS

This study highlights the extent of stress and depression among Canadian women during a critical time period for infant development. It also provides evidence for the "healthy immigrant effect" for recent immigrants but points to an elevated risk for depression during pregnancy and the postnatal period for immigrant women who have settled in their new homeland for 5 to 10 years.

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ABSTRACT #7

Determinants and variability of docosahexaenoic acid (DHA) content in human milk in the CHILD Study: implications for allergic disease

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BACKGROUND
An adequate supply of dietary Docosahexaenoic Acid (DHA) (C22:6, omega-3 fatty acid) is critical for human nutrition. Low DHA intake during infancy has been associated with an increased risk of chronic diseases including asthma and allergies [1, 2], and the DHA content in breastmilk varies widely between mothers [3, 4]. We aimed to characterize this variation according to maternal diet and other demographic, environmental and health factors in a national cohort of Canadian women.

METHODS
We studied a representative sub-group of 400 mothers participating in the Canadian Healthy Infant Longitudinal Development (CHILD) Study from four sites across Canada (Vancouver, Edmonton, Manitoba and Toronto). A food frequency questionnaire administered during pregnancy was used to document maternal diet and estimate DHA intake. The DHA content of breastmilk samples collected at 3 months postpartum was determined by gas chromatography and expressed as percentage total fatty acids (%TFA). Linear regression models were used to determine associations between DHA content, estimated DHA intake and other maternal characteristics.

RESULTS
The median DHA content in breastmilk among all mothers was 0.12%TFA (interquartile range: 0.09%– 0.19%) and the median estimated dietary DHA intake was 95mg per day (interquartile range: 41mg– 170mg), originating mainly from fish and fortified dairy or egg consumption. DHA in breastmilk was strongly correlated with dietary DHA intake ($r = +0.30$, $p<0.001$). Use of daily maternal multivitamins or fish oil supplements also predicted higher DHA in breastmilk (both $p<0.001$). Independent of dietary DHA and supplement use, DHA in breastmilk was significantly lower among obese mothers, and significantly higher among mothers with a postsecondary degree, those of Asian ethnicity, and those residing in Vancouver. In a multivariate model adjusting for all of these dietary and non-dietary factors simultaneously, all were significant independent predictors of breastmilk DHA. Maternal age, asthma, allergy, and season of milk collection were not associated with DHA content in breastmilk.

CONCLUSIONS
Our results confirm that maternal diet and supplement use are key determinants of DHA content in breastmilk, and further identify several non-dietary factors that may influence the intake of this important bioactive nutrient among breastfed infants in Canada. These findings may be relevant to nutrition-based allergic disease prevention strategies. Ongoing research in the CHILD Study will extend analyses to evaluate additional fatty acids in breastmilk and explore associations with child health outcomes throughout early childhood.

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ABSTRACT #8

Examining the role of TLR2 in regulating cow's milk allergy

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BACKGROUND
Oral tolerance is a state of unresponsiveness of the immune system to food antigens. Failures of tolerance induction can lead to future allergic reactions. Milk allergy, is the most common childhood allergy [1]. Beta-lactoglobulin (βLG) is a major allergen in cow's milk. Several factors, such as transforming growth factor-beta, vitamin A, and soluble toll-like receptors (sTLRs) that are found in breast milk are thought to enhance oral tolerance toward breast milk-transferred antigens. In contrast, we have shown previously that PAM_3CSK_4, a TLR2 activator, is able to disrupt oral tolerance toward ovalbumin in mice. The variable levels of these tolerogenic or sensitizing factors in breast milk or baby formulas might be critical to the development of tolerance or allergy. The objective of this work was to better understand the impact of milk derived factors in the development of tolerance.

METHODS
Models of oral tolerance to cow's milk, βLG, and ovalbumin were established. Oral tolerance was assessed in wild type and TLR2-deficient mice through analysis of antigen-specific antibody levels after a systemic antigen challenge. The development of antigen-specific Tregs and tolerogenic dendritic cells were also assessed. sTLR2 levels in milk products were analysed by ELISA.

RESULTS
Oral administration of doses of skim milk or βLG induced the development of oral tolerance against βLG, independent of TLR2. IgE levels in antigen fed mice were 35% less than control (n=15). In contrast to ovalbumin, tolerance induction to βLG in milk was not altered by the addition of PAM_3CSK_4. Soluble TLR2 was observed in cow's milk and in commercial baby formulas. Milk was also shown to enhance the development of ovalbumin-specific Treg cells and tolerogenic dendritic cells in the mesenteric lymph node.

CONCLUSION
Our results confirm an important role for immune components of milk in regulating tolerance and highlight the role of sTLR2, which could inform both allergy prevention and treatment strategies.

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ABSTRACT #9

Immune genes are differentially methylated in relation to early life stress exposure

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BACKGROUND
Changes in an individual's immune system in response to early life stress have important implications for the lifetime health of an individual. One potential mechanism through which early life stress could become biologically embedded to effect immune function is epigenetics. Here we have performed an epigenome-wide association study on the effects of early life stress and adversity measures on DNA methylation, to examine which genomic pathways show differential methylation.

METHODS
DNA methylation was interrogated with the Illumina Infinium HumanMethylation450 BeadChip [1] and genotyping was done on the Psych Chip [2]. Genetic ancestry was called using identical by shared state (IBS) clustering in the statistical program Plink (version 1.07) [3]. DNA methylation analysis was performed on all probes on the array, using linear mixed effects modeling. Enrichment of Gene Ontology terms in the list of differentially methylated genes was tested using overrepresentation analysis was done ErmineJ [4].

RESULTS
In an epigenome-wide analysis of the effects of early life stress we found genes involved immune related pathways were enriched for differential methylation.

CONCLUSIONS
We have seen that early life adversity embeds a distinct signal on the epigenome based on the source of the early life stress. However, multiple forms of stress we have examined share a signal of differential methylation in immune genes.

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ABSTRACT #10

DNA methylation profiles unique to a longevity region: Nicoya, Costa Rica

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BACKGROUND
Aging is an inevitable biological process, involving several molecular and physiological changes, which exhibit substantial heterogeneity across individuals. The Nicoya Peninsula in Costa Rica represents a population having one of the highest demographically validated old age life expectancies in the world [1]. The increased lifespan has been hypothesized as a result of environmental exposures and lifestyle factors such as nutrition, exercise, and social status; however, the reasons for this longevity remain uncharacterized from the perspective of underlying biological mechanisms. DNA methylation is a process that is responsive to environmental influences and has the potential to regulate gene expression, with particular trends that are highly correlated to chronological age [2]. Given the different environmental exposures these populations experience, DNA methylation is most likely acting as a possible mediator between Nicoyan individuals and the longevity observed.

METHODS
We assessed DNA methylation of whole blood in 95 Costa Ricans, aged between 60-110 years old, using the Illumina 450k Methylation Array. Blood cell proportions were estimated using DNA methylation using a well established reference based method [3].

RESULTS
We identified a significant difference in the DNA methylation-based predicted proportion of two age-associated cell types: CD8+ naïve and CD8+ memory T cells. Interestingly, CD8+ naïve T cells decrease with age, whereas CD8T+ memory cells increase. The proportion of CD8+ naïve cells was higher and CD8+ memory T cells was lower in Nicoyan samples, respectively, when compared to control samples from surrounding non-longevity areas of Costa Rica (CD8T+ naïve: p value <0.01, CD8T+ memory: p value < 0.05).

CONCLUSIONS
Results may represent a product of environmental influence that may explain this suggested immunologically younger phenotype in Nicoya. In summary, by DNA methylation profiling we were able to detect a biological distinction of cellular abundance that may potentially be representative of a slow-aging marker in an ethnically novel and unique centenarian cohort.

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ABSTRACT #11

Longitudinal measures of DNA methylation associated with alcohol exposure cessation in purified T-lymphocytes

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BACKGROUND
Previous studies have demonstrated that lifestyle factors such as cigarette smoking and heavy alcohol consumption are associated with differential DNA methylation in peripheral blood [1-3]. However, examination of intervention-based, longitudinal measures of these effects remain limited [4]. Here we aimed to assess genome-wide differences in DNA methylation following an intervention-based cessation of alcohol exposure in purified T-lymphocytes.

METHODS
Twenty-four Caucasian men were recruited for a 3 week intervention study involving a cessation of excessive alcohol consumption. Magnetic bead separation was used to isolate CD3-positive T-lymphocytes from pre- and post-intervention peripheral blood mononuclear cell (PBMC) samples of participants, as well as 23 healthy male controls that were matched for age, ethnicity and smoking behaviour. DNA methylation was measured at over 450,000 CpG sites genome-wide in these samples using the Illumina HumanMethylation450K Beadchip array.

DNA methylation data was subjected to quality control processing, normalization and correction for technical variation. Principal component analysis (PCA) was used to investigate associations to global DNA methylation patterns and linear regression analyses were used to identify site-specific DNA methylation alterations.

RESULTS
Smoking (measured as cigarettes per day) and alcohol abuse status were associated with top-ranking principal components, collectively comprising ~20% of the variance in the DNA methylation data. Linear regression analyses with paired testing identified site-specific DNA methylation differences between pre- and post-intervention samples. Interestingly, these intervention-based DNA methylation alternations had minimal overlap with the differences seen in the healthy control samples.

CONCLUSIONS
Intervention-based cessation of alcohol use is associated with longitudinal site-specific DNA methylation differences.

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ABSTRACT #12

Integration of Transcriptomics, Proteomics and Genome-wide Association Studies (GWAS) with NetworkAnalyst to Gain Insights into Innate Immunity

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BACKGROUND
Advances in high-throughput omics technologies allow us to quantify global cellular changes in DNA, RNA, proteins or metabolites under various experimental conditions or disease states. One active area of research is to develop methodology for integrating these large datasets to improve our mechanistic understanding and provide systems-level insights into cellular function. Furthermore, data integration can increase our statistical power as well as removing study bias. Here, we present NetworkAnalyst [1,2], an interactive, web-based tool for biologists to perform complex meta-analysis, visual data mining and data integration.

METHODS
There are two common approaches to data integration: (1) horizontal data integration, which combines different data sets from the same omic approach, such as performing meta-analysis of gene expression changes to identify expression signatures; and (2) vertical data integration, which seeks to coalesce different omic data sets, such as integrating transcriptomic and metabolomics data, to identify perturbation in pathways of interest. To link these different datasets, NetworkAnalyst mines InnateDB [3] for curated protein-protein interactions (PPI) and creates biologically meaningful networks. Network topology analysis can be used to identify those protein nodes that are highly connected (i.e. hubs), which can serve as useful biomarkers or therapeutic targets; while densely connected units (i.e. modules) that show strong differential expression patterns can be used to identify unique disease signatures.

RESULTS
Using NetworkAnalyst, we can generate novel hypotheses on various aspects of immune regulation, from neonatal vaccination response to common mis-regulation that contributes to inflammatory diseases such as asthma, vascuilitis and sepsis. For instance, we typically identify mis-regulated modules containing S100 protein family members in patients with inflammatory disorder. In patients who developed sepsis, we observed up-regulation in S100A8 and S100A9 genes and down-regulation in S100A4 gene [4]. A number of these S100 proteins have emerged as potential biomarkers in independent studies [5].

In contrast, we used NetworkAnalyst to overlay proteomic data onto transcriptomic networks to characterize neonatal response to vaccination. We observed the up-regulation of a key immune hub, STAT1, in our transcriptome analyses. Independently, we identified known STAT1 PPI in our proteomic analyses. Together, our data suggest STAT1 may play an important role in immunization response against viral vaccines.

CONCLUSIONS
Biological network analysis is a powerful approach to study complex diseases, and can be an invaluable tool for biomarker discovery and the identification of targets for therapeutic interventions.

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ABSTRACT #13

Impact of the hospital microbial environment on infant's gut microbial composition at 3-4 months

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BACKGROUND
Hospital acquired infections (HAIs) affect 8% of Canadian children, with higher prevalence rates found in infants 12% [1]. In Canada, the average length of hospital stay after vaginal delivery is about 2 days but post caesarean delivery it is 4 days [2]. An extended hospital stay increases infant exposure to the hospital microbial environment and elevates the risk for gut colonization with opportunistic microorganisms at a time when the seeding of intestinal microbiota in infants is critical in shaping their immune system. Asthma and atopic disease has been associated with gut microbiota dysbiosis during infancy.

OBJECTIVE
To determine the impact of infant exposure to the hospital environment on the composition of gut microbiota.

METHODS
A subset of 787 fecal samples from the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort from Edmonton, Winnipeg and Vancouver sites were included in this study. Data on infant hospital length-of-stay at birth and afterwards was obtained from birth chart reviews and maternal report at 3 months postpartum. Infant gut microbiota at 3-4 months was characterized by Illumina 16S rRNA sequencing. Microbial relative abundance, Shannon diversity and Chao1 species richness were determined. The gut microbial profile of infants hospitalized for more than 1 day after birth (n=557,70.8%) was compared to the profile of infants without this hospital exposure with the Mann-Whitney U-test.

RESULTS
Newborn exposure to the hospital environment for more than one day was associated with higher fecal abundance, at infant age 3-4 months, of microbes belonging to the *Lachnospiraceae* family (p=0.041). Moreover, in a comparison analysis restricted to vaginally delivered and not treated with antibiotic at birth (both directly and indirectly), the *Pseudomonadaceae* (p=0.04) and genus *Pseudomonas* (p=0.027) were more abundant in the gut of infants with extended hospital stays after birth. This dysbiosis was evident in both vaginally and caesarean-delivered infants.

CONCLUSION
This study highlights the association between extended exposure to the hospital microbial environment after birth and changes to infant gut microbial composition, including greater colonization with hospital-acquired pathogens, within the first 3 months of life. The impact of these compositional changes on the development of gut immunity and atopic disease later in life requires further study.

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ABSTRACT #14

Maternal depression during pregnancy and 4-month infant gut immunoglobulin A levels

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BACKGROUND
Secretory immunoglobulin A (sIgA) has a critical role in early life gut mucosal immunity and is a marker of immune maturation. Delayed IgA production is associated with increased risk of allergic diseases [1]. Animal studies of stressful events before birth and during infancy show changes in the vaginal microbiome, as well as changes in intestinal microbial composition and lower sIgA concentrations in offspring [2, 3]. A first report in humans found infants born to mothers with greater stress during pregnancy more likely have gut dysbiosis [4], but there is a paucity of literature on stress-microbiome-immunity pathways in humans. This study investigated differences in total infant fecal IgA levels at 4 months according to the depression status of the mother before or during pregnancy.

METHODS
The data were obtained from a sub-sample of 47 term infants from the Vancouver and Winnipeg sites of the Vanguard cohort of the Canadian Healthy Infant Longitudinal Development (CHILD) Study. Mothers of the infants were enrolled during pregnancy and were asked to report depression previously or currently through a standardized questionnaire. Pre/postnatal stress and depressive symptoms were ascertained from scored-scales administered to the general CHILD cohort for comparison. Infant stool samples were collected at a mean age of 3.9 months, and total fecal IgA was measured using an enzyme-linked immunosorbent assay. Mann-Whitney U-tests were used to detect differences in IgA levels using IBM SPSS version 23.

RESULTS
Five trajectories for depressive symptoms and perceived stress in mothers (chronic, antepartum, postpartum, never-high scores, never-low scores) during pregnancy and at postpartum were found in the general cohort. About 8% of women had depressive symptoms during pregnancy. In the Vanguard cohort, 32% of mothers reported depression before or during pregnancy and 11% during pregnancy only. There was no statistically significant difference in fecal IgA between infants born from depressed and not depressed mothers (p=0.63) in the Vanguard cohort; however, the median IgA in infants of mothers that were depressed is lower than in infants of not depressed mothers (10.9 (IQR = 4.5-27.8) vs. 13.5 (IQR = 7.2-36.4)ug/g of total protein).

CONCLUSIONS
Infants born to mothers with depression before or during pregnancy appear to have lower fecal IgA levels at 4 months. The small sample size and measurement of total IgA in place of sIgA may have prevented detection of statistical significance.

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ABSTRACT #15

Maternal depression during pregnancy and fecal short chain fatty acid levels in infants

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BACKGROUND

Maternal stress and depression can affect the composition of gut microbiota in infants. Gut microbiota produce large quantities of active metabolites, such as butyrate and other short-chain fatty acids (SCFA). Changes in microbial composition that alter metabolite levels have the capacity to influence host intestinal cells, including those of the immune system. SCFAs have recently been associated with anti-inflammatory activity not only in the gut, but also in peripheral tissue, such as the lungs [1]. We hypothesized an association between maternal depression and altered infant fecal SCFA levels which might induce allergic inflammation in offspring. This study investigated differences in infant fecal SCFA levels at 4 months according to the depression status of the mother during and after pregnancy.

METHODS

This was a sub-study of 240 mother-infant dyads from the Edmonton, Vancouver and Winnipeg sites of the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort. The CES-D (Center for Epidemiologic Studies Depression) questionnaire was administered to women at recruitment (mean 27 weeks) and 36 weeks of gestation, and postnatally at 6 months, 1 year, 1.5 years and 2 years. Based on CES-D scores, pre and postnatal trajectories for maternal depression were identified. Short chain fatty acid (SCFA) concentrations (acetate, butyrate, propionate, valerate) were measured by NMR as mmol/L in fecal samples obtained from infants at a mean age of 3.8 months. Statistically significant associations between maternal depression trajectory membership and infant fecal SCFA levels were tested by ANOVA using SPSS version 23.

RESULTS

Five trajectories for maternal depressive symptoms during and after pregnancy (chronic, antepartum only, postpartum only, never-high scores and never-lower scores) were identified in the CHILD cohort. Women in the postpartum trajectory had comparable CES-D scores during pregnancy to women in the never depressed trajectory. Fecal butyrate levels in infants born to mothers in the antepartum depression trajectory were numerically higher (mean 1.71 mmol/L, N=12, $p<0.06$) than in infants of mothers in the postpartum depression trajectory (mean 0.77 mmol/L, N=12), and of mothers with never-lower depression scores during and after pregnancy (mean=1.05 mmol/L, N=143, $p<0.07$).

CONCLUSIONS

We report evidence of a tentative association between maternal depression during pregnancy and higher levels of butyrate produced by microbiota in fecal samples of infants at 4 months of age.

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ABSTRACT #16

Linking the indoor microbiome with atopy in the CHILD study: preliminary findings

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BACKGROUND
Microbial communities of the indoor built environment have long been studied, largely using culture techniques; however, recent studies employing next-generation community sequencing analyses have suggested that indoor microbial communities are much more diverse and complex than previously thought [1]. How the make-up of the indoor microbiome affects the long-term health of the individuals within is not entirely clear.

METHODS
Samples of household dust were obtained from 80 homes in Winnipeg, Canada, occupied by a selection of subjects enrolled in the Canadian Healthy Infant Longitudinal Development (CHILD) Study when the children were 3-4 months of age. Skin prick testing for sensitization to common food and environmental allergens and standardized physician's diagnosis for atopic dermatitis was obtained for each child in the study at 1 and 3 years of age. For each dust sample, community bacterial partial 16S rDNA was sequenced using MiSeq (Illumina). Simpson's diversity indices of variable groups were compared by Kruskal-Wallis test using False Discovery Rate to correct for multiple test comparisons.

RESULTS
Dust samples were dominated by members of the phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria, with *Staphylococcus*, *Corynebacterium*, and *Streptococcus* as the highest-abundance genera. The bacterial diversity was generally higher in homes of children who were reported to have atopic dermatitis or were sensitized to food allergens on skin testing. Further, we observed significant differences ($p < 0.05$) in the bacterial diversity of the dust in homes of children with *vs* children without: atopy (1 year p=0.018 and 3 years p=0.038) and food sensitivity (1 year p=0.018 and 3 years p=0.004).

CONCLUSION
These results provide preliminary findings that reveal a link between the microbiome of the indoor environment and allergic health outcomes early in a child's life. We recognize the N for the allergic groups is small. Therefore these results highlight the need for funding to further analyze the dust microbiome to increase power and provide a clearer snapshot of the entire CHILD cohort.

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ABSTRACT #17

Early life intestinal microbial alterations are associated with pediatric asthma in rural Ecuador

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BACKGROUND

Asthma is the most prevalent chronic disease among children and affects 235 million people worldwide [1]. Although the incidence of asthma in South America is among the highest worldwide, underlying causes and disease phenotypes are poorly defined and may differ to developed countries. Recent evidence in mice [1] and human [3] has identified a 'critical window' early in life where the effects of gut microbial changes (dysbiosis) are most influential in immune development and experimental asthma. Given the differences in gut microbiota between North and South American populations, we aimed to validate our previous work in a microbially-different human population.

METHODS

We compared the gut microbiota of 97 infants from the coastal community Las Esmeraldas, Ecuador at 3 months of age by 16S sequencing (V4 region) Illumina MiSeq. Subjects were grouped into atopic-wheezers (n=27) and controls (n=70) based on a skin prick test and wheezing history at 1 year of age. An exact logistic regression model was developed to evaluate the risk associated with the AW group according to specific clinical and epidemiological metadata. Metagenomes were predicted from 16S rRNA OTU data using PICRUSt, and categorized by function using KEGG Orthology. The concentration of fecal short chain fatty acids (SCFA) was determined by gas chromatography.

RESULTS

Atopy and wheezing at 1 year of age was significantly associated with asthma diagnosis at 5 years (OR 17.8), birth by C-section (OR 3.1), potable water at home (OR 2.7) and in utero exposure to antibiotics (OR 2.9). This phenotype was also significantly associated with eosinophil concentration at 2 and 5 years ($P < 0.05$), number of episodes of respiratory infections ($P < 0.01$), maternal load with *Trichuris trichiura* during pregnancy ($P < 0.05$), and inversely associated with the number of diarrheal episodes by 1 year of age ($P < 0.05$). Similar to what we had previously found in Canadian babies, atopic-wheezing Ecuadorian babies also exhibit gut microbial dysbiosis at 3 months of age. However, the microbial alterations were different and more pronounced in Ecuadorian babies. Predicted metagenomic analysis showed significant differences in genes involved in carbohydrate and taurine metabolism. Fecal acetate was significantly reduced in atopic wheezers. Ongoing experiments will determine if differences in eukaryome are also associated with asthma risk in this population.

CONCLUSIONS

This study further supports the importance of the microbiota during the first 100 days of life, although the characteristics of the microbial dysbiosis and epidemiological associations depend on geographical location. Reduced fecal acetate as a common feature in both populations suggests that different microbial alterations may have similar metabolic outcomes.

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ABSTRACT #18

Postnatal exposure to household cleaning products shape the infants' gut microbiota composition at 3-4 months

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BACKGROUND
According to Health Canada, the average Canadian family uses 20-40 litres of cleaning products each year [1]. In developed countries there is accumulating evidence of an increased risk for asthma and atopic disease with exposure to household cleaning products [2-5]. Infant gut microbiota dysbiosis has been linked to asthma and allergic disease. Hence, the aim of this study was to assess if the gut microbial composition of infants is affected by postnatal exposure to household cleaning agents.

METHODS
The study involved 787 infants enrolled in the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort from Edmonton, Winnipeg and Vancouver sites. At 3 months after delivery, mothers were asked to complete questionnaires on aspects of their health, environment, lifestyle and personal use of household cleaning products namely: disinfectant (multi-surface cleaner), detergent and other chemicals (spray air-freshener). Fecal samples were collected at 3-4 months and fecal microbiota were characterized by Illumina high-throughput sequencing of the hyper-variable V4 region of the 16S rRNA gene. Bacterial taxon abundance, microbiota richness and diversity were compared between infants with high indoor disinfectant exposure (n= 414, 52.6%) and low indoor disinfectant exposure (n=373, 47.4%) based on above and below median of total frequency scores, using Mann-Whitney U-test. Comparisons were corrected for multiple testing with the False Discovery Rate and stratified to adjust for confounding factors, such as infant antibiotic treatment.

RESULTS
More than half of the CHILD cohort households used disinfectants at least once a month, mostly multi-surface cleaner. At 3-4 months of infant age, high indoor disinfectant exposure was associated with low fecal abundance of Actinobacteria(p=0.0002) at the phylum level and of *Bifidobacteriaceae*(p=0.021) at the family level. A similar trend was detected in a comparison restricted to vaginally delivered, exclusively breastfed infants not treated with antibiotics (directly and indirectly). Moreover, total microbial diversity was reduced at the order level (p= 0.028) in infants in the high exposure group. Additionally, *Pseudomonas* was more abundant in infants exposed to low disinfectant and high other chemicals compared to low disinfectant and low other chemicals (p= 0.012).

CONCLUSION
Our findings find evidence of an association between household disinfectant exposure and changes to infant gut microbial composition at 3-4 months of age. They suggest the possibility that cleaning products may affect development of the infant gut microbiome and immunity, with possible consequences for allergic disease later in life.

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ABSTRACT #19

Adverse long-term consequences associated with a history of asthma in patients with chronic obstructive pulmonary disease: an observational population cohort study

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BACKGROUND
It has been shown that patients with COPD and concurrent asthma have a poor prognosis compared with asthma or COPD alone. However, existing longitudinal evidence is limited and conflicting. We performed an observational population cohort study using provincial health administrative data to evaluate long-term adverse health consequences in subjects with COPD that was and was not preceded by asthma.

METHODS
We used health administrative data from 1991 to 2013. Individuals with newly physician diagnosed COPD between ages 40 and 55 years were identified by a COPD health administrative case definition of 1 or more COPD hospital discharges and/or 3 or more COPD ambulatory care visits between April 1, 2009 and March 31, 2011. The date the definition criteria were met was the index date. Individuals were followed from the index date to March 2013. The primary outcome was all-cause mortality or COPD hospitalization, whichever occurred first. The exposure was a history of asthma at least two years before the index date to ensure that it preceded COPD. Demographics, rural and income status, region of residence, comorbidities at baseline and exposure to health care before baseline were considered as confounders. Cox proportional hazards models were used to evaluate an association between a history of asthma and long-term consequences in COPD patients. To adjust for confounding, the analysis was performed on a weighted sample with weights assigned using propensity scores.

RESULTS
9,053 patients were included in the main analyses: a median age of 50 years at COPD diagnosis, 51% males. 2,717 (30%) patients had a history of asthma. COPD patients with a history of asthma were more likely to be female, have a higher level of comorbidity at baseline and be exposed to health care compared to COPD patients without a prior asthma history. Over a median follow-up time of 2.9 years, 1,126 (12%) subjects experienced an outcome of interest. After adjustment for confounding, having a history of asthma was significantly associated with increased risk of the composite outcome (HR=1.22, 95% CI: 1.06-1.40; Table 1).

Table 1. Association of a history of asthma and all-cause mortality or COPD hospitalization in patients with COPD tested with Cox regression analyses.

Exposure	Hazard Ratio (95% Confidence Interval)
Univariate analyses	
History of asthma	1.23 (1.09-1.39)
Multivariable analyses (variables adjusted for: sex, age at COPD diagnosis date, income, immigrant and rural status, region of residence, prior comorbidities (prior diabetes, hypertension, cardio-vascular diseases, gastroesophageal reflux disease, mental health, atopic conditions, Aggregated Diagnosis Groups [ADG] categories), prior exposure to primary, specialist care and flu vaccination	
History of asthma	1.19 (1.04-1.35)
On weighted sample based on the propensity score estimated using generalized boosted regression modeling (adjusted, reweighted average treatment effect on the treated [ATT] estimate)	
History of asthma	1.22 (1.06-1.40)

CONCLUSIONS

In a large population of patients with newly diagnosed COPD, a history of asthma was significantly associated with an increased risk of all-cause mortality and COPD hospitalization.

ACKNOWLEDGEMENTS

ABSTRACT #20

Predictors of inappropriate usage of rescue medications in asthma: a 12-year population-based study

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BACKGROUND

Despite strong evidence of harm, patients continue to receive excessive rescue medications such as short acting beta agonist agents (SABA). Understanding factors associated with the inappropriate use of SABA (IUoS) can help develop better policies to tackle this problem.

METHODS

We used British Columbian (BC) administrative health data of between 2002 and 2013. We created a retrospective cohort of individuals between 14 and 55 years of age based on a validated asthma definition. The follow-up time was divided into adjacent 12-months periods. The outcome of interest was IUoS, as defined in a previously published algorithm [1]. Exposures were demographic variables at baseline and indicators of types and quality of care (fee code for spirometry, care provider specialty) as well as appropriate use of inhaled corticosteroid (ICS) in the prior year [2]. A generalized linear model was used to examine exposure-outcome associations, controlling for several potential confounding variables.

RESULTS

352,936 individuals (56% female, average age 30.2 at entry) satisfied the case definition, generating 2.6 million patient-years. On average, 18.5% of patient-years were categorized as IUoS. The factor most strongly associated with IUoS was appropriate use of corticosteroid; (OR=0.30, 95% CI 0.29-0.30). Female sex (OR=0.95, 95%CI 0.94-0.97) and increasing age was associated with lower odds of inappropriate use, and increasing age OR=0.96, 95% CI 0.96-0.97) was associated with a lower likelihood of inappropriate use. Among quality of care indicators, use of spirometry was associated with 5% reduction in the odds of IUoS. Patients with a history of a visit to specialists including respiratory medicine, internal medicine, and allergy/immunization in the previous year showed 29%, 22% and 14% lower likelihood of IUoS, respectively (Table 1).

Table 1: Results of the regression analysis of inappropriate use of SABA

Variable Group	Variable	Odds Ratio	95% CI (Lower, Upper)	P value
Patient characteristics	Sex (female=1)	0.95	0.94-0.97	<.0001
	Socio economic score	0.98	0.97-0.98	<.0001
	Year	0.95	0.95-0.95	<.0001
	Age (per 10 years increase)	0.96	0.96-0.97	<.0001
Type & quality of care (Asthma attributable)	Pulmonary function test	0.95	0.93-0.98	<.0001
	Ratio of ICS to total medication more than 50%	0.30	0.29-0.30	<.0001
	Respirologist consultation	0.71	0.68-0.74	<.0001
	Cardiologist consultation	1.73	0.88-3.39	0.110
	Internal medicine consultation	0.78	0.75-0.81	<.0001
	Allergist consultation	0.86	0.82-0.89	<.0001
	General Practitioner visits			
	No visit	0.56	0.55-0.56	<.0001
	1 visit PY (Reference group)	-	-	-
	2 visits PY	0.77	0.76-0.78	<.0001
	More than 2 visits	0.53	0.52-0.54	<.0001
	Continuity of care (COC)			
	COC=0 (Reference group)	-	-	-
	COC>0 and COC<50%	0.84	0.82-0.85	<.0001
	COC>=50% and COC<100%	0.83	0.82-0.85	<.0001
	COC=100%	0.85	0.83-0.87	<.0001
Comorbidity (Asthma attributable events are excluded)	Number of hospitalizations	1.01	1.00-1.02	0.143
	Number of physician visits	0.88	0.87-0.89	<.0001
	Modified Charlson score	1.00	0.99-1.01	0.130

CONCLUSIONS
Despite proven safety issues, inappropriate use of SABA still occurs among a significant minority of asthma patients, necessitating further research into its determinants. Several factors related to patient and process/quality of care are associated with inappropriate use of SABAs. Guidelines have not been successful in ensuring appropriate asthma treatment especially among patients under the care of primary care providers.

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ABSTRACT #21

What do MRI ventilation defects reveal about asthma control?

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BACKGROUND
Improving asthma control is a primary treatment goal for patients with severe asthma. Recent studies using multiple-breath-gas-washout techniques have suggested that poor asthma control may be related to ventilation heterogeneity [1, 2]. We previously determined the relationship between magnetic resonance imaging (MRI) ventilation abnormalities and asthma severity, but the links between asthma control and ventilation defects have not been ascertained. Thus, here our objective was to determine the relationship between ventilation defects, visualized using MRI, and asthma control. We hypothesized that MRI ventilation defects would be related to biomarkers of asthma control.
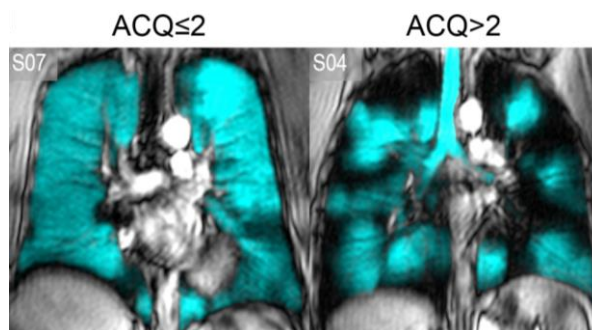
METHODS
Volunteers with severe asthma provided written-informed-consent to an ethics-board-approved protocol and underwent spirometry, multiple-breath-nitrogen-washout (MBNW) and noble gas MRI during a single two-hour visit. Asthma control and quality of life were evaluated using the Asthma-Control-Questionnaire (ACQ) and Asthma-Quality-of-Life-Questionnaire (AQLQ). Ventilation heterogeneity was quantified using the lung-clearance-index (LCI) and MRI ventilation-defect-percent (VDP) [3]. Univariate relationships were evaluated using Pearson correlations (r). Unpaired t-tests were performed to compare ventilation heterogeneity in subjects stratified by ACQ (≤ 2 or >2) and AQLQ scores (≥ 5 or <5) and previous 6-month exacerbation history (<1 or ≥ 1). All statistical analyses were performed using GraphPad Prism version 6.02 (GraphPad Software Inc.; La Jolla, CA, USA).

RESULTS
We evaluated 18 severe asthmatics (46±12 years) with poorly-controlled disease (ACQ=2.3±0.9). Mean VDP was 12±11% and LCI was 10.5±3.0. Both VDP (p=0.008) and LCI (p=0.02) improved post-bronchodilator. Whereas VDP was strongly correlated with LCI (r=0.86, p<0.0001), the post-bronchodilator change in VDP and LCI was not correlated (p=0.08). As shown qualitatively in Figure 1, there was a greater number and volume of MRI ventilation abnormalities in participants with worse ACQ. There was also significantly worse VDP but not LCI in asthmatics with ACQ >2 (p=0.04) and AQLQ<5 (p=0.04). There was a trend towards worse VDP (p=0.053), but not LCI (p=0.3), in asthmatics reporting ≥ 1 exacerbation in the past 6-months. VDP (but not LCI, p=0.052) was correlated with ACQ (r=0.62, p=0.01) but not AQLQ or exacerbation history.

Figure 1. Hyperpolarized ^{3}He MRI of two representative patients with severe asthma. Centre coronal slice ^{3}He MRI ventilation in blue co-registered to anatomical ^{1}H MRI in grey-scale for two representative severe asthmatics. S07 ACQ$\leq$2: 55 year-old female, FEV$_1$=77%$_{pred}$, ACQ=1.4, LCI=9.7, VDP=2%; S04 ACQ>2: 60 year-old female, FEV$_1$=47%$_{pred}$, ACQ=4.3, LCI=14.0, VDP=35%.

CONCLUSIONS
MRI ventilation defects were significantly worse in patients with worse asthma control and quality of life. This is important because regional MRI ventilation defects may be considered as a sensitive intermediate endpoint of asthma control. Such ventilation abnormalities can be rapidly and serially quantified to help guide treatment in order to improve asthma control.

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ABSTRACT #22

Answering a 130 year old question for asthma and airway fibrosis using multimodal nonlinear optical microscopy

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BACKGROUND
Current methods for measuring the morphological changes that occur with disease within the lung are performed by histological staining of tissue sections, which is usually very time-consuming and not always effective [1]. An emerging optical modality, multimodal nonlinear optical (NLO) microscopy, has shown potential and utility in label-free visualization of key extracellular molecules [2, 3] involved in tissue remodeling, such as collagen and elastin. The objective of this study is to quantify the disease-related morphological changes of collagen and elastin at specific tissue sub-regions within the asthmatic airway based on imaging directional and texture analysis.

METHODS
Lungs of asthmatic and non-asthmatic subjects not suitable for transplantation were donated for research through the International Institute for the Advancement of Medicine (IIAM, USA). Tissue cores were sectioned and donors from both genders with ages ranging from 5 to 47 years old were investigated using an in-house built multimodal femtosecond nonlinear optical microscope which is capable of acquiring co-localized collagen and elastin images of airways samples simultaneously.

RESULTS
Abnormal thickening of the airway basement membrane is one of the hallmarks of airway remodeling in asthma. Representative images showing this region under the NLO microscope are presented in Figure 1. The thickening of the basement membrane observed in Figure 1 has been proposed to lead to a defect in cellular communication between epithelial and mesenchymal cells which together form the epithelial-mesenchymal trophic unit, leading to airway remodeling. Thus it is important to understand the variations in structure of the extra-cellular matrix components (*i.e.* collagen and elastin) with the basement membrane and how this relates to cellular functions. Changes in collagen and elastin fibers morphology such as fiber length, fiber thickness, fiber alignment are tracked by texture analysis [4] of NLO images. Among other parameters, the entropy of NLO images, for example reveals a highly–directional and organized collagen and elastin fiber morphology in the non-asthmatics compared to a less-organized collagen/elastic structure characterized by shorter but denser fiber bundles in the asthmatic airways. Collagen morphological changes were further explored and it was observed that changes occur at the fibril level. As shown in Figure 1, collagen fibrils were imaged by transmission electron microscopy (TEM), and they present significant structural differences (p=0.0017) when comparing airways from asthmatics and non-asthmatics donors.
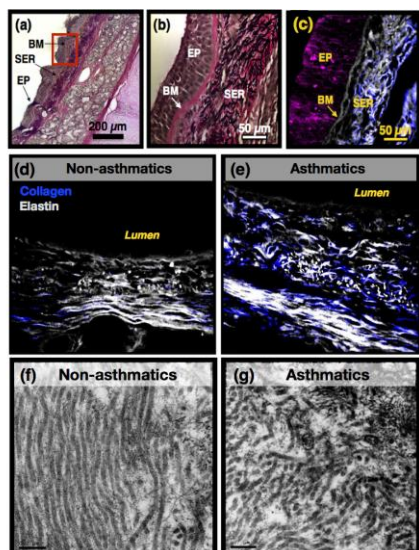
Figure 1 Examples showing Verhoeff Van Giessen stained (a and b) tissue as well as an overlay of two label-free NLO images (c) acquired at the lamina propria region. Elastic fibers are shown in black and collagen is shown in brown-yellowish colour (a and b). Elastic fibers imaged with TPEF are shown in gray/white (c, d and e); collagen fibers, imaged by SHG are shown in blue (c, d and e). Examples of TEM images of collagen fibrils are shown in (f) and (g). It is possible to notice that both collagen fibers and fibrils present different morphology. BM: basement membrane; EP: epithelium; SER: sub-epithelial region (lamina propria).

CONCLUSIONS
This preliminary study is pioneering the identification of structural changes that occur during asthma development based on NLO microscopy and imaging analysis. It provides novel insights to researchers from other areas to better understand the mechanisms of collagen/elastin deposition in airways, showing structures with a level of details never achieved before.

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ABSTRACT #23

Defective collagen I remodeling and contraction is a feature of asthmatic airway fibroblasts

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BACKGROUND
Asthma is a chronic inflammatory disease of the airways that is associated with airway remodelling that involves all tissues of the airway wall including the epithelial, sub-mucosal, and smooth muscle layers as well as vascular structures [1]. In particular, accumulation of fibroblasts and deposition of excess collagen in the laminar propia is a well documented feature of airway remodeling in asthma [2]. In this research, we studied the ability of fibroblasts from asthmatic and non-asthmatic airways to remodel collagen I using optical imaging methods.

METHODS
Airway fibroblasts were obtained from donor lungs deemed not suitable for transplant from both asthmatic (n=10) and non-asthmatic (n=10) individuals. Cells were cultured in 10% FBS in DMEM with added antibiotics/antimycotics and seeded between passage 3 and 5 onto collagen I gels. Gel contraction over time was then quantified as a percentage of initial gel area at 72 hours using ImageJ software. Collagen fiber formation was assessed using multiphoton microscopy and transmission electron microscopy. Gels were also analyzed for protein levels using western blot analysis.

RESULTS
It was determined that seeding 37,500 cells per well provided a measurable gel contraction rate which could be recorded between 0-72 hours. When comparing the ability of airway fibroblasts to contract collagen I, we found asthmatic derived fibroblasts were unable to contract collagen I gels as well as non-asthmatic derived airway fibroblasts (p=0.017). Multiphoton analysis revealed that non-asthmatic airway fibroblasts were able to remodel gelatin into fibrillar collagen I compared to asthmatic derived airway fibroblasts (p=0.033). This finding was further confirmed using transmission electron microscopy which demonstrated fibril and fibre formation of collagen I within the gel assays.

CONCLUSIONS
Our data suggest that asthmatic fibroblasts are deficient in their ability to produce and remodel collagen compared to fibroblasts derived from non-asthmatics. This study helps to expand our understanding of collagen deposition and remodeling in asthma and creates potential opportunities for therapeutic intervention.

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ABSTRACT #24

Exploring rare coding and non-coding variants reveals new genes associated to lung obstruction

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BACKGROUND
Asthma is a complex inflammatory trait that has an important genetic and environmental component. In the past years, genome-wide association studies (GWAS) allowed to identify many genes associated to the trait but explained only a small fraction of the genetic risk [1]. Those results led the motivation to delineate the whole genetic architecture of the disease, since GWAS are only exploring the common single nucleotide polymorphisms (SNPs). One of the GWAS limitations, rare variants, has been explored in asthma in a few studies [2, 3]. However, those focused only on the coding part of the genome [2, 3]. Here, we explored both coding and non-coding regions to assess the impact of rare variants in asthma related traits.

METHODS
A custom capture panel of coding and non-coding regulatory regions followed by next-generation sequencing was applied to 149 trios (447 individuals) from the Saguenay—Lac-Saint-Jean asthma familial collection. Single variant association test and collapsing tests (combined multivariate and collapsing test (CMC), variable-threshold approach (VT) and SNP-set (sequence) kernel association test (SKAT)) were performed for asthma related continuous traits (forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), Tiffeneau-Pinelli index (FEV_1/FVC), histamine provocative concentration causing a 20% drop in FEV_1 (PC_{20}) and IgE levels). Only single nucleotide variations (SNVs) were included in the analysis and variants with minor allele frequency of <5% were included in the collapsing tests. Kinship coefficient, age and sex were used as covariates.

RESULTS
Significant associations are only observed for FEV_1/FVC. Four rare SNVs are associated after single variant association test, of which three are located in the non-coding region of the genome. Those rare variants are located in the vicinity of the RNU6-2/ARHGAP5 and CD36/GNAT3 genes, and in the ADAMTS2 gene. For the collapsing tests, variants were combined together based on their closest gene. One gene was significantly associated using SKAT, twelve using VT and eight CMC. The gene RNF8 was significant in all three tests and six were significant in both CMC and VT: ALKBH3, DJCA, MIR5186, PANDAR, MLIP-IT1 and SIT1. The gene ADAMTS2 was also significant after VT test.

CONCLUSIONS
Exploring rare variants associated to continuous traits linked to asthma allowed to identify genes newly associated to lung obstruction. By including coding and non-coding variants, our study led to the identification of genes that would have been missed if only considering coding regions.

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ABSTRACT #25

Comparative outcomes of the Nasal Allergen Challenge model of the Allergic Rhinitis Clinical Investigator Collaborative versus the Environmental Exposure Unit

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BACKGROUND

The Allergic Rhinitis Clinical Investigator Collaborative uses a Nasal Allergen Challenge (NAC) model to evaluate the clinical efficacy of novel therapeutics for allergic rhinitis (AR) and to study the cellular mechanisms and pathobiology of AR. Previous pilot studies have optimized the qualifying criteria and allergen concentration used in the NAC model. In the current study, we aimed to compare subjective nasal symptoms and objective nasal airflow measurements obtained with the NAC model to results from an Environmental Exposure Unit (EEU) study.

METHODS

Eleven birch allergic individuals who had participated in a study validating the use of birch pollen in the EEU were screened, with six completing the study. During screening, four-fold increases in birch allergen concentration from 1:128 were delivered intranasally every 15 minutes until a fall in Peak Nasal Inspiratory Flow (PNIF) of $\geq 50\%$ from baseline and a Total Nasal Symptom Score (TNSS) $\geq 8/12$ were obtained. The cumulative allergen concentration of all doses received at screening was used as the concentration to be delivered during the NAC visit. Three non-allergic participants were also enrolled. During the NAC visit, participants recorded their PNIF and TNSS at baseline (before challenge) and 15 minutes, 30 minutes, 1 hour, and hourly until 12 hours post-challenge. Nasal symptom scores from the EEU study were obtained for each participant and GraphPad Prism was used for the statistical analysis.

RESULTS

An immediate sharp increase in TNSS was observed following NAC, with participants reaching peak TNSS 15 minutes post-challenge ($p<0.01$). By contrast, in the EEU study, a gradual increase in TNSS was observed, with participants reaching peak TNSS after 3.5 hours of continuous controlled birch pollen exposure ($p<0.0001$). The % PNIF reduction mirrored these observations, with participants reaching peak % PNIF reduction at 30 minutes ($p<0.05$) and 4 hours ($p<0.05$) during the NAC study and EEU exposures, respectively. Both the NAC and EEU were able to evoke a significant increase in TNSS ($p=0.0003$ for NAC, $p<0.0001$ for EEU) compared to baseline. A moderate ($R^2=0.58$, $p=0.02$) and strong ($R^2=0.83$, $p=0.0006$) correlation between TNSS and % PNIF reduction was observed for the NAC and EEU respectively.

CONCLUSIONS

Even though both the NAC and EEU are able to evoke nasal AR symptoms, the kinetics of this AR response differ between the two models. Industry partners should consider these differences when choosing an optimal allergen challenge model to use for the evaluation of their novel AR therapeutic agent.

ABSTRACT #26

Investigating differential expression patterns of complement system-related genes in individuals with allergic asthma

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BACKGROUND
Allergic asthma is a chronic inflammatory disorder of the airways, often characterized by biphasic airway contraction in response to controlled inhalation of allergens. The molecular mechanisms underlying the difference between an early phase responder (ER), who experiences an isolated bronchoconstriction within minutes following the allergen exposure, and a dual phase responder (DR), who additionally experiences a chronic bronchoconstriction several hours after the initial exposure, remain elusive. As a danger sensing component of the innate immune system, activation of complement system is strongly associated with the disease [1]. In this study, the effect of complement system-related genes on allergic asthma was investigated and validated. We hypothesize that complement system-related genes are differentially expressed in ERs and DRs.

METHODS
Peripheral whole blood samples were collected using PAXgene Blood RNA tubes from mild asthmatic and non-asthmatic subjects. 8 complement system-related genes were previously identified in a discovery cohort of 29 subjects with RNA-sequencing. NanoString nCounter Elements assays were applied to the same discovery cohort and additional subjects (n=42, ER=16, DR=26), as well as another cohort (n=64, ER=4, DR=52, Control=8) to validate the findings. Differential expression analysis was used to identify significant genes.

RESULTS
With a Benjamini-Hochberg false discovery rate cutoff of 0.1, SERPINA1, PLAUR and C5AR1 were down-regulated in DRs of the discovery cohort and additional subjects. No significant differences in gene expression were found between ERs and DRs in the validation cohort; however, F13A1, CD46, C5AR1, SERPINA1, PLAUR, CD59 and C3AR1 were up-regulated in asthmatic individuals in comparison to non-asthmatic controls.

CONCLUSIONS
The complement system plays an important role in allergic asthma, as well as in the late phase response of allergic asthma. More ERs are needed to further validate the results.

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ABSTRACT #27

Effect of inhaled allergen challenge on eosinophil and neutrophil activation in subjects with mild allergic asthma following inhaled allergen challenge

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BACKGROUND

Eosinophils and neutrophils migrate to the site of allergic stimulation and contribute to airway hyper-responsiveness and airway remodelling through the release of toxic granule proteins, reactive oxygen species, cytokines and lipid mediators [1,2]. Our understanding of systemic activation of these cells following allergic stimulation of the airways is limited. This study evaluated the expression of activation markers on circulating eosinophils and neutrophils, and levels of their degranulation products, eosinophil peroxidase (EPX) and neutrophil elastase (NE), collected before and after whole lung allergen inhalation challenges (AIC) in mild allergic asthmatics.

METHODS

Eleven subjects with mild allergic asthma were challenged with inhaled allergen and developed early and late phase asthmatic responses. Blood and sputum samples were collected at baseline, 7 and 24 hours following AIC. Blood cells were stained with antibodies to identify neutrophil (CD45+, CD16+) and eosinophil (CD45+, CD16-) populations, and activation was determined by expression of CD11b, CD18, CD35, CD62L, CD63, and CD66b [3, 4]. Sputum samples were processed using dithiothreitol (DTT). EPX and NE levels in sputum supernatant and plasma samples were measured by ELISA. GraphPad Prism was used to perform 1-way ANOVAs and Tukey post-hoc analyses; data are presented as mean±SEM.

RESULTS

At 7 hours post-challenge there was a significant increase in eosinophil expression of CD18 (1.6-fold), CD62L (1.8-fold) CD63 (1.6-fold), and a significant increase in neutrophil expression of CD35 (2.27-fold) ($p < 0.05$). At 24 hours post-challenge there was a significant increase in eosinophil expression of CD18 (1.9-fold), CD35 (1.2-fold), CD62L (2.3-fold), and a significant increase in neutrophil expression of CD18 (2.04-fold) and CD63 (1.83-fold) ($p < 0.05$). Plasma levels of EPX were significantly lower at 7 hours (134.6±5.10 ng/mL) and 24 hours (129.9±3.30 ng/mL) post AIC compared to baseline (150.1±5.09 ng/mL; $p < 0.05$). There were no statistically significant changes in plasma and sputum supernatant levels of NE and sputum supernatant levels of EPX at 7 and 24 hours following AIC compared to baseline.

CONCLUSIONS

We demonstrate that circulating eosinophils and neutrophils respond similarly in their upregulation of some markers of activation following a whole lung AIC. This shared response may be beneficial when developing therapies targeting granulocytes for treatment of asthma. In contrast to other reports, EPX levels decreased slightly in the circulation post-challenge, and this decrease may suggest lung-homing of these cells [5]. No increase of sputum EPX or NE levels following AIC contrasts previous reports, and may reflect the method of sample processing and poor stability of proteins during storage.

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ABSTRACT #28

Airway epithelial cells reduce airway smooth muscle cell contractility

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BACKGROUND
Airway smooth muscle (ASM) plays a critical role in modulating airway diameter. ASM cells are considered to exist in one of two phenotypes at any given time; contractile or proliferative [1]. Co-culture of ASM with airway epithelial cells has been reported to induce proliferation but effects on the contractile properties are unexplored [2]. The aim of the current study was to examine whether ASM cells cultured in the presence of epithelial cells have a reduced contractile phenotype.

METHODS
Primary ASM and normal human bronchial epithelial cells (NHBE) were harvested from control subjects. Epithelial cell line, BEAS-2B, was also utilized. Epithelial cells cultured on Transwell® permeable supports were placed in culture with ASM for 24 hours before calcium or force responses to histamine were measured by fura-2 imaging or traction force microscopy respectively. Protein and RNA was collected for analysis by Western blot and qPCR.

RESULTS
ASM co-cultured with epithelial cells demonstrated a reduction in force generation and calcium release after stimulation with 1μM histamine. Co-cultured cells had reduced mRNA and protein of specific contractile apparatus proteins. We observed no change in the expression of caveolin-1, or the expression of mRNA governing proteins responsible for calcium release, including CD38, IP_3R, PLCβ or SERCA. COX-2 and mPGES-1 mRNA were increased after co-culture, suggesting a possible phenotype switch towards a pro-inflammatory state. Pre-treatment with the COX-1 inhibitor indomethacin and SC-560 restored calcium signaling after histamine stimulation.

CONCLUSIONS
Airway epithelial cells cause a reduction in ASMC contractility *in vitro* that is not transcriptionally regulated. Further examination of COX-2 products may provide explanations for this observed phenotype.

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ABSTRACT #29

IgE+ B cells increase in the airways following whole lung allergen challenge in mild allergic asthmatics

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BACKGROUND
In allergic diseases, exposure to allergen drives isotype switching in B cells to become IgE-switched B cells [1]. IgE-switched B cells further differentiate into IgE-producing plasma cells through an IgE+ plasmablast intermediate or through an IgE+ memory B cell. Allergen exposure has been demonstrated to induce IgE class switching in the airway of asthmatics [2], but the resulting B cell response is poorly understood. We previously demonstrated that IgE+ memory B cell subsets in circulation (blood), hematopoeitic (bone marrow) and lymphatic (tonsil) compartments remained unchanged following allergen inhalation challenge [3]. This study evaluated IgE+ memory B cell subsets in the airways (sputum) of allergic asthmatics following whole lung allergen challenge.

METHODS
Seven subjects with mild allergic asthma were recruited for whole lung allergen challenges and spirometry was measured for 7 hours after allergen inhalation. Sputum was induced by inhalation of 3%, 4% and 5% hypertonic saline, and sputum cells were isolated and stained with CD19, CD45, IgE and IgG. Cells were acquired using a Becton Dickinson LSRII flow cytometer, and analyzed using FlowJo software. Lymphocytes were defined as $SSC^{low}CD45+$, and B cells were defined as $SSC^{low}CD45+CD19+$. GraphPad Prism was used to perform 1-way ANOVAs and Tukey post-hoc analyses. Data were expressed as median (range) unless indicated otherwise.

RESULTS
Inhaled allergen induced a mean±SEM maximum fall in FEV_1 of 30.2±4.4% within 2 hours post-allergen and 10.2±3.7% 3-7 hours post-allergen. B cell levels measured in the airways were 6 (1-12)% at baseline, 23 (4-28)% 7 hours post-allergen, and 9 (6-21)% 24 hours post-allergen. IgE+ B cell levels were 11 (4-20)% at baseline, 39 (17-57)% 7 hours post-allergen, and 14 (13-41)% 24 hours post-allergen. The allergen-induced increase in B cells and IgE+ B cells 7 hours post-allergen were statistically significant ($p<0.05$). Total and allergen-specific IgE were also detectable in sputum, and total IgE significantly increased 7 hours post-allergen ($p<0.05$).

CONCLUSIONS
Whole lung allergen challenges in subjects with mild allergic asthma increased the levels of IgE+ B cells and total IgE in the sputum. The data suggests that antigenic stimulation of the airways leads to enhanced airway levels of IgE+ B cells and IgE, which contributes to the pathogenesis of allergic asthma.

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ABSTRACT #30

Diesel Exhaust and Allergen Co-exposure Enhances Mucin Secretome in Human Airways

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BACKGROUND
Diesel exhaust (DE) is the most significant source of urban ultrafine traffic-related air pollution. In a controlled human exposure study, DE exposure has been demonstrated to augment allergen-induced airway inflammation [1]. However, tissue specific changes driving the synergy from allergen-DE co-exposure remains poorly elucidated.

METHODS
We performed a randomized, double-blinded, controlled human crossover study. Five mild asthmatics, sensitized to the study allergen, inhaled filtered air or DE (300 mg/m^3) for 2 hours. Subsequently, one lung segment was challenged with allergen, and saline was administered to a contralateral segment. Bronchoalveolar lavage (BAL) and endobronchial biopsies were obtained 48 hours after allergen challenge. This was repeated 4 weeks later, but for opposite inhalation and new segments for the allergen challenge. Four experimental conditions were derived: FAS (filtered air and saline), DES (diesel exhaust and saline), FAA (filtered air and allergen) and DEA (diesel exhaust and allergen). BAL from five subjects were pooled for each condition and processed by LC-MS/MS using a label-free quantitative proteomics approach. Endobronchial biopsies from five subjects were immunostained for different mucins (MUC), such as Muc5AC, Muc4 and Muc16.

RESULTS
Proteins were selected with >99% confidence criteria (*i.e.* at least two non-redundant peptides identified with a log(e)≤3). The resulting expression matrix contained more than 2500 proteins across all four conditions. Expression intensity was determined by spectral counting. Relative fold changes in FAA, DES and DEA were calculated compared to FAS. Co-exposure with DEA significantly altered the expression of 76 proteins compared to FAA and 130 proteins compared to DES. These studies showed that Muc5AC, Muc4 and Muc16 were elevated more than eight-fold with DEA co-exposure compared to either allergen or DE exposures alone. Muc5B was detected at similar levels with DE alone and DEA co-exposure. None of the mucins detected were enhanced by allergen alone, whereas Muc2 and Muc7 were detected only in the DEA secretome and not in any of the other three conditions.

CONCLUSIONS
This is the first study to demonstrate that DE and allergen co-exposure enhances specific glycoproteins in the lungs, which may be associated with increased induction of mucus production. Changes in the mucus composition following DE-allergen co-exposure may contribute to the synergistic effect of DE exposure on allergen-induced airway inflammation.

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ABSTRACT #31

Climate change, asthma and allergy risk in Toronto

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BACKGROUND
Plant pollen and fungi spores of select species contribute to asthma and allergic exacerbations and may cause these chronic illnesses to develop [1]. These organisms interact with the climate to influence production, timing and dispersal of their airborne glycol-proteins. Our aim is to describe past associations among weather, pollen, spores, allergies and asthma in Toronto, Canada in order to gain a better understanding of future trends in a changing climate.

METHODS
Time-series analysis will be used to measure temporal associations between health encounters, multiple genera of aeroallergens and weather, and place the findings in the context of regional climate projections. Various time lags will be incorporated to account for the complex relationships among weather and biota. Exposure estimates for the metropolitan population of 2,615,060 (2011) are derived from daily pollen and spore counts from air monitoring between 2004 and 2014. The pollen exposure estimate is assigned to individuals who access health services for allergic rhinitis (AR) and asthma. Covariates include age, sex, socio-economic status and air pollution. Geographic information will be used to add spatial variation to the exposure estimate by describing the distribution of birch trees, a plant with known allergenic properties. Additional allergenic species' ranges will be estimated and overlain to generate a single continuous raster surface of pollen exposure. Health outcome data collection is not complete, but utilizes International Classification of Disease (ICD) and Ontario Health Insurance Plan (OHIP) codes for AR and asthma encounters for the exposure time period.

RESULTS
Time-series for pollen, spores and weather data have been calculated for 2004-2014. Results suggest well-defined seasonality of the pollen seasons, which begin in the spring with trees, followed soon after by grasses and lastly, by weeds. Preliminary results describing temporal associations between health encounters and environmental data are expected to be ready by the conference date.

CONCLUSION
The findings of this research will contribute to the risk assessment of climate and health vulnerabilities undertaken by the City of Toronto. We hope to identify opportunities for illness prevention as pollen seasons are extended and precipitation and temperature patterns for fungal ecology shift with climate change.

ACKNOWLEDGEMENTS
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ABSTRACT #32

Impacts of environmental exposures on incidence and trajectories of childhood asthma

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BACKGROUND
In a population-based birth cohort, the Border Air Quality Study (BAQS), an initial investigation on pediatric asthma in early years showed an association between perinatal exposure to Traffic Related Air Pollution (TRAP) and asthma incidence in the first years of life [1]. We extended this investigation to include co-varying exposures, namely green spaces and TRAP, in a larger sample that we followed up during the first 10 years of life.

METHODS
65,254 children born between 1999 and 2002 in Metropolitan Vancouver were followed until age 10, using linked administrative health databases. Asthma cases were sex- and age-matched to five randomly chosen controls. Associations between (a) different metrics of TRAP, (b) satellite-derived measure of green spaces, and asthma incidence during preschool (0-5 years) and school age (6-10 years) periods were estimated with conditional logistic regression. In an effort to better understand the course of asthma, trajectories of asthma phenotypes were determined using group-based trajectory modeling [2]. Furthermore, we examined the impact of air pollution exposure and green spaces on the various clusters of asthma phenotypes using weighted multinomial regression.

RESULTS
The investigation of incident asthma showed that air pollution was associated with the odds of asthma in children during the ages between birth and age 5 (adjusted Odds Ratio using interpolation method per interquartile exposure increase (95% CI): NO: 1.06 (1.01 - 1.11), NO2: 1.09 (1.04 - 1.13)), CO: 1.05 (1.01 - 1.1)), but not at older ages [3]. These associations with air pollution during the preschool period were partially offset by the independent protective effect of the level of residential greenness surrounding the home location (0.96 (0.93–0.99)) [4]. Finally, we described two trajectories of children with chronic asthma that were significantly associated with NO_2 exposure: those who developed the disease starting in early infancy and those who started to show healthcare utilization for asthma treatment and/or diagnosis around age 5 or 6, coinciding with the cut-off year between preschool and school-age time periods that were examined in the incidence analysis, where low-term birth weight children were identified as a vulnerable subgroup.

CONCLUSIONS
Pregnant mothers' exposure to TRAP exposure is likely a risk factor for development of asthma, this effect is most prominent among children who developed the disease in early infancy.

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ABSTRACT #33

Polydimethylsiloxane as versatile passive air sampler for measuring levels of phthalates indoors

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BACKGROUND
Phthalates are plasticizers used in a variety of consumer building products including vinyl flooring and wallpapers, and personal care products such as cosmetics. Exposure to phthalates that are released from these sources have been linked to increased risk of asthma, allergies and other auto-immune diseases [1]. A step towards limiting human exposure to phthalates is to measure their abundance. Active air samplers are conventionally used for this purpose but their use is hindered by cost, need for electrical power, skilled operation and their noise [2]. Passive air samplers (PAS), which work by diffusion, are efficient alternatives to active samplers. They are cheap, noise-free, have no power and minimal skill requirements [2]. We have developed a PAS from commonly available rubber, polydimethylsiloxane (PDMS). PDMS collects and retains a wide range of chemicals and is easy to use [3]. Owing to its versatility, PDMS can be used for stationary sampling of micro-environments, monitoring human personal exposure and even exposure of wildlife. This project was aimed at developing a versatile and easily used passive air sampler.

METHODS
PDMS stationary samplers were deployed in 22 Toronto homes for one month in the summer of 2013. In a personal exposure study, two participants wore PDMS broaches in their breathing zone while working at their desks for 8 hours daily for a total of 4 days, in the fall of 2015. Post-deployment samples were extracted and analysed for phthalates using gas chromatography mass spectrometry (GC-MS).

RESULTS
Average concentration of $\sum_6$ phthalates ranged from 1153±421 to 1351±556 ng/m^3 in bedrooms and living rooms, respectively. No significant difference ($p > 0.01$) was found between bedroom and living room levels, indicating the ubiquitous and well-mixed nature of phthalates and indoor air, respectively. PDMS worn as a personal passive air sampler was able to detect phthalates after 1 day.

CONCLUSION
This study shows the feasibility and versatility of using PDMS as a passive air sampler for measuring levels of phthalates in the indoor environment.

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ABSTRACT #34

Cellular mechanisms involved in the allergic lung response to the environmental bioaerosol archaea *Methanosphaera Stadtmanae*

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BACKGROUND

Bioaerosols in occupational environments are associated with the development of inflammatory lung diseases [1]. The archaea specie *Methanosphaera stadtmanae* (MSS) is found in high concentrations in poultries, dairy farms and swine confinement buildings bioaerosols (up to 10^8 archaea/m^3) [2-4]. In mice, MSS induces an inflammatory lung response characterized by T cells, eosinophils, neutrophils, and IgG production [5]. However, in order to better understand the potential impact of MSS exposure on the pulmonary health of workers, further characterization of the immunopathology induced by this archaeon is essential.

METHODS

Wild-type (WT) mice were exposed to either 3µg (low dose) or 100µg (high dose) MSS by intranasal instillation, three consecutive days a week for three weeks. Four days after last exposure, mice were euthanized. The immune response polarity was determined by flow cytometry analysis of T_H cell populations (T_H1, T_H2 and T_H17) and by the evaluation of antigen-specific serum immunoglobulins. Lung airway hyperresponsiveness was measured using a Flexivent apparatus. To study possible mechanisms involved in the response, inflammation severity was analyzed in ILC2-, mast cell-, eosinophil-deficient, $Cd34^{-/-}$ and $Tlr4^{-/-}$ mice exposed to MSS using broncho-alveolar lavages cell counts.

RESULTS

In WT mice, 3µg MSS induced a weak T_H2 response and a strong T_H17 lung response, characterized by IgG_1 (but not IgG_{2a} and IgE) production. Interestingly, high doses of MSS led to a lower eosinophil counts and full polarization into a T_H17 response. Moreover, at low MSS dose, mice did not develop airway hyperresponsiveness. Using transgenic mice (eosinophil- and mast cell-deficient), we found that eosinophils, mast cells, and ILC2s cells are not required for the inflammatory response to MSS. No differences were found in $Cd34^{-/-}$ mice, indicating no important role for this molecule in the response. $Tlr4^{-/-}$ mice exposed to MSS had a strong decrease in airway inflammation compared to WT mice. However, as we found endotoxins in our MSS lot, part of the phenotype observed in $Tlr4^{-/-}$ mice could be attributable to endotoxin activity.

CONCLUSIONS

We conclude that MSS exposure induces a T_H17-dominated inflammatory lung response which could be harmful to workers exposed to environments where high levels of MSS and endotoxin are present in bioaerosols.

ACKNOWLEDGEMENTS

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ABSTRACT #35

Lung clearance index is elevated in asthmatic children with symptomatic control

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BACKGROUND

Lung Clearance Index (LCI), a measure of global ventilation distribution inhomogeneity derived from multiple breath washout (MBW) testing, has been shown to be sensitive in the detection of early cystic fibrosis lung disease [1]. However, very little data exists on its utility in the assessment of childhood asthma. This study aims to determine whether LCI is elevated among preschool and school age children with well-controlled asthma who are asymptomatic at the time of assessment.

METHODS

A total of 36 children (ages 3-16 years) diagnosed with asthma and 89 healthy controls of similar age were enrolled. All asthmatic children were prescribed controller therapy and reported symptom control on questionnaires. Both groups performed MBW testing using 4% sulfur hexafluoride as the tracer gas and an AMIS 2000 mass spectrometer (Innovision A/S, Odense, Denmark) and custom data acquisition and analysis software, followed by spirometry using the Vmax Encore system (CareFusion, USA). Spirometry z-scores and percent predicted values were calculated using the Global Lung Initiative equations. The upper limit of normal (ULN) for LCI was calculated using data from the healthy control group.

RESULTS

In both asthmatics and healthy controls, 81% of patients were able to complete spirometry. Mean FEV_1 and FEV_1/FVC ratios were within normal range and comparable between asthmatics and healthy subjects. Mean ($\pm$ SD) LCI was significantly higher in asthmatic patients compared to healthy controls (6.70$\pm$1.15 vs. 6.16$\pm$0.36, p=0.003). These were well controlled asthmatics, as evidenced by the fact that they had no bronchodilator response in spirometry. Sixteen asthmatic patients (44%) had an LCI value above the ULN despite reporting symptom control. Amongst asthmatic patients unable to perform spirometry, 6/7 (86%) had an abnormal LCI (>ULN) value.

CONCLUSIONS

LCI was found to be elevated amongst asymptomatic asthmatic children who were prescribed controller therapy. Longitudinal studies are needed to define the clinical significance of an elevated LCI in terms of its predictive value for subsequent asthma exacerbation or persistence of asthma into adulthood. Further studies need to be done to assess the value of LCI in monitoring of asthma control.

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ABSTRACT #36

The effects of birth weight on infant pulmonary function

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BACKGROUND

Given the importance of the prenatal environment on organogenesis [1], we hypothesized that birth weight as a manifestation of the prenatal environment may have impacts on infant lung function beyond the initial measurement and may play a role in long-term respiratory health. In this study, we have examined the impacts of birth weight on infant lung function in a sub-cohort of a general population birth cohort study, the Canadian Healthy Infant Longitudinal Development (CHILD) Study to further understand the role of prenatal environment on lung health.

METHODS

Pregnant women recruited to the Toronto site of the CHILD Study were invited to participate in an infant lung function study. Pulmonary function tests included multiple breath washout (MBW) testing, from which we derived the Lung Clearance Index (LCI), performed on three occasions, early, mid and late infancy. To understand the role of the birth weight, infants with weights in the upper centile (>90th, *i.e.* 4000g) and lowest centile (<10th, *i.e.* 2800g) were compared to those within these centiles. Linear mixed effects models with random intercept were used to assess the effects of birth weight on lung function measurements.

RESULTS

We obtained pulmonary function values from 218 (30% of the total Toronto cohort) participants (58% males and 42% females) during infancy, of which 32 completed one visit, 106 completed two visits and 80 completed three visits. Data from all visits were included in analysis. These participants were on average (standard deviation) 9.07 (3.92) months old at their first visit, and had length-for-age z-score and weight-for-age z-score of -0.28 (1.24) and 0.02 (0.99), respectively. Twenty-five (11%) of participants were classified as overweight and 18 (8%) of participants were classified as underweight at birth. The LCI was on average (Standard Error) 0.26 (0.11) units (p=0.02) higher in those infants that were born overweight, after adjusting for age, height and gender (Figure 1). No effect of weight <10% percentile on LCI was observed. This trend is consistent when using LCI z-score as the outcome, with 0.56 (standard error=0.2, p=0.006) units higher in those overweight infants.
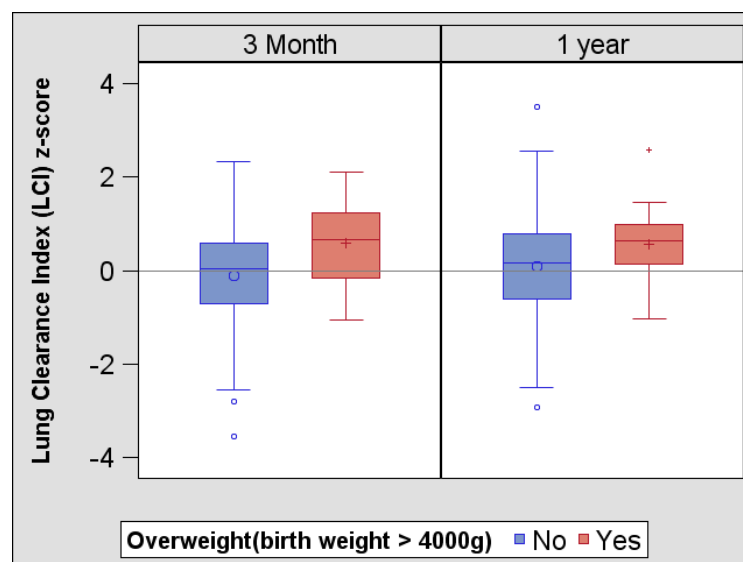

Figure 1. Relationship between birth weight and LCI measured at 3 months and 1 year

CONCLUSIONS

The prenatal environment, as manifested by birth weight, has a significant influence on lung function, as measured by LCI, in the first year of life.

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ABSTRACT #37

Ventilation inhomogeneity in asymptomatic infants with a history of recurrent wheezing

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BACKGROUND
The care of children with recurrent wheezing relies largely on clinical assessment, as objective measures of lung function are currently lacking. The Lung Clearance Index (LCI), measured during multiple breath washout (MBW), is a sensitive marker of early airway disease in children with cystic fibrosis (CF), but has not been explored in infants with recurrent wheezing (RW). We aimed to compare LCI values amongst infants with a prior history of RW to healthy controls as well as infants with CF.

METHODS
Thirty-seven (37) infants with RW, 35 CF infants and 113 healthy infants, asymptomatic at time of testing, underwent MBW measurement using an AMIS 2000 mass spectrometer and 4% SF_6 as a tracer gas. Infants with RW or CF were recruited from outpatient clinics; whereas healthy control children were tested as part of the Canadian Healthy Infant Longitudinal Development (CHILD) Study.

RESULTS
After adjustment for age and height, there were overall significant differences in mean LCI across the three groups (P=0.007). Subsequent multiple comparisons indicated that RW infants had LCI values reflecting increased ventilation inhomogeneity despite being tested during a period of clinical stability compared with healthy infants (7.22±0.15 *vs.* 6.70±0.08, p=0.01). There were no significant differences in any other pulmonary function parameter, *e.g.* forced expiratory volume in 0.5 seconds ($FEV_{0.5}$), forced vital capacity (FVC), forced expiratory flow 25-75% of expiration (FEV_{25-75}) or functional residual capacity (FRC_{pleth}) to differentiate RW children. A greater proportion of RW infants, 35% (13/37) had abnormal LCI values (> 95 percentile of the normal range) compared with CF infants, 20% (7/35). Infants with abnormal LCI values had concordantly lower $FEV_{0.5}$ and higher FRC_{pleth} than children with an LCI in the normal range (p<0.05). Amongst RW infants, higher exhaled nitric oxide values (36 *vs.* 22 ppb, p=0.06) and parental reported poor steroid response were more likely to be associated with abnormal LCI measurement (p=0.05).

CONCLUSIONS
Ventilation inhomogeneity is present in clinically stable infants with RW. Children identified with abnormalities in LCI had consistent abnormalities in other lung function tests. Clinical symptoms were not predictive of abnormalities in LCI. Raised LCI may characterize a novel infant severe wheezing phenotype.

ABSTRACT #38

Lung Clearance Index (LCI) in 3 year old children with clinically assessed preschool asthma

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BACKGROUND
Asthma diagnosis in preschool children is complicated by its phenotypic overlap with common transient wheezing. As a result, there is an emphasis on predicting the persistence of symptoms into school age. Clinical predictions currently employ models limited by modest sensitivity [1], namely the Asthma Predictive Index (API), and may benefit from the addition of objective lung function measures. The Lung Clearance Index (LCI) is a measure of ventilation inhomogeneity, derived from Multiple Breath Washout (MBW) tests [2]. Previous data has correlated elevated LCI with persistent asthma and multi-trigger wheeze [3]. However, no data exists for LCI in mild asthmatics from the general community. Here, we report LCI measurements from the Canadian Healthy Infant Longitudinal Development (CHILD) Study, a general population birth cohort.

METHODS
Participant data was obtained from the CHILD Study Toronto general cohort. During 3-year visits, a subset of participants performed MBW SF_6 testing, as previously described [4]. Quality control was completed per ATS/ERS guidelines [5]. LCI values were included for healthy controls (HC) and all definite and possible (D&P) preschool asthmatics, as defined by clinical assessment. The HC group had confirmed absence of respiratory symptoms, congenital respiratory problems, and parental smoke exposure. Population demographics explored sex, age, height, and weight, with applicable z-score calculations. Parametric t-tests and Wilcoxon rank sum tests were used to compare mean LCI measurements between groups.

RESULTS
At the 3-year visit, acceptable LCI measurements were produced for 101 participants, of which 67 were HC and 34 had definite (n=6) or possible (n=28) asthma. Mean LCI (standard deviation) was 6.07 (0.43) and 6.42 (0.78) for HC and D&P asthma, respectively. LCI was an average of 0.35 (95%CI:[0.06, 0.63]) units higher in D&P asthma than in healthy controls (P=0.02). 3 definite and 6 possible asthmatics had an abnormal LCI (defined >95th percentile). API positivity was not helpful in predicting an abnormal LCI (P=0.67). We were unable to detect a significant difference in LCI between total API positive (n=9) and API negative (n=25) asthmatics (P=0.188).

CONCLUSIONS
LCI is elevated in 3-year old children with clinically assessed D&P asthma compared to healthy controls. Future longitudinal studies will determine whether elevated LCI is predictive of persistent asthma at 5 years.

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ABSTRACT #39

Ventilation inhomogeneity in severe acute wheezing preschool children as measured by nitrogen based multiple breath washout

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BACKGROUND
Multiple breath nitrogen washout (N2MBW) and its primary outcome measure, Lung Clearance Index (LCI), is a sensitive measure of ventilation inhomogeneity (VI) and has potential utility in younger populations where spirometry is not as feasible. Furthermore, LCI has been used as a monitoring tool for evaluating preschool asthma control. Understanding how LCI changes during acute episodes is essential to furthering the use of MBW in asthma diagnostics and management.

METHODS
N2MBW was attempted in preschool subjects recruited from the emergency department (ED) at SickKids Hospital who were discharged with physician diagnosed asthma, and had received salbutamol during their visit. Baseline visits occurred within 5 days of discharge; follow-up visits occurred within 10-14 weeks of the baseline visit. N2MBW was performed using an Exhalyzer D System (EcoMedics AG, Switzerland). MBW was performed per ATS/ERS recommendations, though only 2 trials were used for a successful test occasion. Statistical analyses were performed using R 3.1.2.

RESULTS
14 subjects in this ongoing study had acceptable baseline results, and 8 of these had acceptable follow-up results. Of these subjects with acceptable results, 8/14 (57%) subjects had abnormal baseline LCI values (defined >95th percentile (LCI = 8.43) of local control cohort of similar age – the Canadian Healthy Infant Longitudinal Development (CHILD) Study, and 1/8 (12.5%) of those subjects had abnormal LCI values at follow-up visits. In those with both baseline and follow-up measurements, mean (standard deviation) LCI was 8.59 (0.97) at baseline, and 7.73 (0.56) at the follow-up visit. This indicates a significant mean decrease in LCI of 0.87(0.86, p=0.024, 95%CI: 0.149, 1.589).

CONCLUSIONS
This study demonstrates that VI is present as measured by LCI in the majority of preschool children in acute episodes of wheezing. The VI improves, but does not normalize in all subjects. However, it was difficult to obtain at least 2 trials for many baseline patients due to their severe wheezing symptoms, indicating that MBW may not be the ideal test in such a severely sick patient group. Further work must be done to understand the factors associated with abnormal LCI at baseline and follow-up and whether the magnitude of change in LCI measurements is related to risk of persistent wheeze.

ACKNOWLEDGMENTS

ABSTRACT #40

Novel approach for the identification of bronchial epithelial cells in lower human airways using flow cytometry

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BACKGROUND
The airway epithelial cell is the initial cell type impacted by inhaled environmental factors, such as pathogens, allergens, and pollutants [1]. Sampling lower airway body fluids like bronchoalveolar lavage (BAL), provides valuable information on the reaction of the lung to inhaled materials [2]. Conventional methods to discriminate bronchial epithelial cells (BEC) in lower airway samples include cytochemical staining, immunohistochemical procedures, standard and confocal microscopy and in-situ hybridization [3]. These techniques present throughput limitations in the number of cells being quantified and time efficiency. Flow cytometry is an important tool that allows delineation of specific cell components of immune responses and disease states [4]. A well-defined flow cytometry panel for the identification of BEC in human lung fluids is currently lacking and we aimed to satisfy the existing need.

METHODS
Human BAL and blood samples were obtained from volunteers enrolled in an ethics-approved clinical study at the Air Pollution Laboratory at The University of British Columbia. Blood samples, BEAS-2B (BEC Cell line) and primary BEC isolated from bronchial brushings were used as negative and positive control respectively. Collection and processing of BAL and blood samples was performed following standard operation procedures in order to lyse and remove red blood cells and clumped tissue.

Cells were examined by side scatter area (SSC-A) versus forward scatter area (FSC-A), then using forward scatter height versus FSC-A to eliminate debris and clumped cells from the analysis. Single cells were sub-gated using Fixable Viability Dye eFluor® 450 and subsequently live cells were discriminated by the expression of CD45 surface marker conjugated to the flourochrome Allophycocyanin/Cy7 (APC-Cy7). Exclusion of CD45 positive cells, commonly used marker for total leukocytes, was followed by the examination of double expression of Fluorescein Isothiocyanate conjugated Pan-Cytokeratin (PanCK-FITC) intracellular marker versus PerCP-Cyanine5.5 conjugated EpCAM (EpCAM PerCP-Cy5.5).

RESULTS
Bronchial epithelial cells collected from human BAL samples were detected by the proposed panel combining intracellular and surface markers. The percentages found in BAL (4.6%), positive controls (92% ±5%) and blood samples (0.0%) were consistent with the literature when other non-flow cytometric methods were used.

CONCLUSIONS
We developed a protocol for the accurate identification and quantification of BEC using flow cytometry.

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ABSTRACT #41

Airway epithelial production of IL-17C in response to bacterial and rhinovirus co-exposure

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BACKGROUND
Human rhinovirus (HRV) is the dominant viral pathogen associated with asthma exacerbations in both pediatric [1] and adult populations [2]. Bacterial pathogens also have been linked to wheezing episodes in children, with asymptomatic bacterial colonization at 1 month being linked to asthma development by age 5 [3]. In adult asthmatics, bacterial colonization has been associated with increased duration of asthma, and exacerbations within the past year. Non-typeable *Haemophilus influenzae* (NTHI) is among the most common bacterial species detected in the airways of severe asthmatic patients [1, 2]. Importantly, bacterial and viral pathogens were commonly detected concurrently during wheezing episodes in children under 3 years old [1]. Further, asthmatic patients who smoke have more poorly controlled disease, and more frequent exacerbations than asthmatics who do not smoke [5]. Bacteria, viruses, and smoke primarily interact with the airway epithelium, but the mechanisms by which they promote asthma development or exacerbations are unclear. IL-17C is a novel cytokine reported to be increased in the bronchial epithelium during NTHI infection, and suggested to have pro-inflammatory and anti-bacterial functions in the airway. The effect of viral exposure on IL-17C has not been examined previously. We hypothesize human bronchial epithelial (HBE) cells initiate IL-17C production in response to respiratory pathogens as a protective anti-microbial mechanism.

METHODS
Confluent primary normal HBE cells were treated with NTHI and/or HRV for up to 24 hours. Other stimuli included replication-deficient HRV, the synthetic double-stranded RNA poly(I:C), and agonists of individual TLRs. Furthermore, HBE cells were exposed to medium or pathogens in the presence or absence of cigarette smoke extract (CSE). IL-17C mRNA and protein levels were measured using qRT-PCR and ELISA.

RESULTS
Neither NTHI, nor HRV-16 alone induced significant IL-17C release. By contrast, co-exposure to NTHI and HRV-16 induced synergistic IL-17C protein release at 24 hours, with mRNA upregulated as early as 6 hours. This response was not HRV serotype-specific as it was observed with both HRV-16 and HRV-1A. Induction of IL-17C was dependent upon viral replication and could be mimicked by poly(I:C). Individual TLR agonists did not synergize with HRV to induce IL-17C. Finally, CSE inhibits the pathogen-induced IL-17C response.

CONCLUSION
Co-exposure to intact NTHI and replicating HRV stimulates synergistic IL-17C protein production in normal HBE cells, and is reduced by acute cigarette smoke exposure. Further studies are needed to clarify the potential role of IL-17C in respiratory infections in the airways of asthmatics and smokers with asthma.

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ABSTRACT #42

Investigating systemic immune responses in peripheral blood of cat-allergic people under the nasal allergen challenge model

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BACKGROUND

Allergic rhinitis (AR), a prevalent disease worldwide, is an IgE-mediated inflammatory condition of the nasal mucosa induced after allergen exposure [1]. Cat allergy affects 10-15% of patients with allergic rhinitis and/or asthma [2]. The standardized nasal allergen (NAC) model may allow us to investigate into AR pathophysiology with biological sampling [3].

METHODS

In this study, 19 participants with a clinical history of allergy to cats underwent NAC. Clinical symptom changes post NAC were measured by Peak Nasal Inspiratory Flow (PNIF) for assessing nasal patency and Total Nasal Symptom Score (TNSS) for participants' symptom recognition. Whole blood was collected into PAXgene tubes at baseline and post NAC (1, 2 and 6 hours). The PAXgene blood lysates were applied to profile 770 immune genes using the nanoString nCounter PanCancer Immune Profiling Panel. The profiled gene expression data was normalized by nSolver (ver. 2.6). The statistical analyses of the complete blood count (CBC) data and the gene expression data were performed using the R statistical computing program {Packages: limma (ver. 3.26.8), nlme (ver. 3.1-125), Mfuzz (ver. 2.30.0)}.

RESULTS

The clinical symptom changes in PNIF and TNSS demonstrated that the NAC had triggered AR response in participants. The comparisons in the CBC and the gene expression data were implemented both at each time point post NAC by comparing to baseline and over time points to identify continuously changed variable. The numbers of lymphocytes and monocytes were significantly changed at 6 hours post NAC compared to baseline and increased over time (P.Value < 0.05). Meanwhile, the number of neutrophils was significantly changed at comparison between each time point (1, 2 and 6 hours) post NAC and baseline (P.Value < 0.05) but not over time. 202 immune genes were significantly differentially expressed over time points (P.Value < 0.05), and the gene expression patterns over time were clustered into 8 groups using the fuzzy c-means algorithm.

CONCLUSIONS

Investigations in peripheral blood collected during NAC identified significant changes in blood cell counts and the whole blood transcriptome in parallel with the onset of AR. Although the investigation wasn't able to cover the whole immune response, it is a cross-section that may provide biomarkers of AR pathophysiology which could be applied to the investigation of mechanisms of action of allergen immunotherapy.

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ABSTRACT #43

The regulation of interleukin-13 receptor subunits in response to injury signals

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BACKGROUND
As a defense barrier, the airway epithelium suffers frequent injury from exposure to environmental challenges and requires repair coordinate with inflammation. Airway epithelial barrier dysfunction and elevated levels of interleukin (IL)-13 are two characteristics observed in asthma. Although IL-13 is known to be a key cytokine in mediating inflammatory and remodelling responses, our group has shown that IL-13 is critical to airway epithelial repair via the release of heparin-binding EGF-like growth factor (HB-EGF) and activation of epidermal growth factor receptor (EGFR). IL-13 signalling is mediated by two receptors, the complex of IL-13 receptor α1 (IL-13Rα1)/ IL-4 receptor α subunit (IL-4Rα) and IL-13 receptor α2 (IL-13Rα2). We hypothesize that the expression of IL-13 receptors is dysregulated in the asthmatic airway epithelium and contributes to the inappropriate IL-13 response observed in asthma.

METHODS
In vitro studies were performed in monolayer cultures of the Human Airway Epithelial (1HAEo-) cell line and primary airway epithelial cells (AEC) from normal and asthmatic donors. Cells were subjected to mechanical wounding or IL-13 stimulation followed by supernatant and cell lysate collection. Cellular distributions of IL-13Rα1/ IL-4Rα and IL-13Rα2 were examined using immunofluorescence and flow cytometry. IL-13 receptor subunits expression was examined in cell lysates via western blotting. Soluble IL-13Rα2 levels in the supernatants were measured via ELISA.

RESULTS
Baseline expression patterns of IL-13 receptor subunits were significantly different between normal donors (primary AEC, 1HAEo-) and asthmatic donors. Both mechanical wounding and IL-13 stimulation altered the cell surface expression of IL-13Rα2 and IL-4Rα over 24 hours while IL-13Rα1 expression remains unaltered in 1HAEo-cells. A significant decrease in the number of membranous IL-13Rα2 positive cells was observed at 30 minutes followed by a significant upregulation at 6 and 24 hours post-treatment. Cell surface density of IL-4Rα was significantly increased at 6 hours post-treatment. At baseline, primary AEC isolated from asthmatic donors express significantly higher levels of IL-13Rα1 and IL-4Rα on the cell surface compared to normal AEC. In response to mechanical injury, IL-13Rα2 expression is upregulated in normal AEC, while asthmatic AEC demonstrate dysregulated expression and function with markedly impaired repair.

CONCLUSION
The regulation of IL-13 receptor expression and trafficking is critical to IL-13 signalling and normal airway epithelial repair. Dysregulated expression of IL-13 receptor subunits may contribute to the abnormal epithelial barrier function in asthma.

ABSTRACT #44

Characterization of IgE receptor expression in human airway epithelial cells

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BACKGROUND
The human airway epithelium serves as the first line of defense against environmental exposures to maintain immune homeostasis. Asthma is an airways inflammatory disease characterized by epithelial barrier dysfunction and cytokine imbalance. Immunoglobulin E (IgE) is known to play a key role in initiating inflammatory responses in asthma. IgE has two receptors, the high affinity receptor, FcεRI and low affinity receptor, FcεRII (CD23). FcεRI is a multi-chain receptor comprising of four polypeptide chains: one alpha (α), one beta (β), two gamma (γ) units. FcεRII (CD23) has two isoforms- CD23a and Cd23b. Expression and distribution of FcεRI and its isomers can contribute to initiating inflammatory responses. The expression of IgE receptors is not well characterized in the airway epithelium. In this study we are examining the expression of IgE receptors in the airway epithelium and investigating their role in mediating allergic inflammation.

METHODS
In an *in vitro* model, monolayer cultures of primary human airway epithelial cells (AEC) from non-asthmatic and asthmatic donors were treated with human and mouse IgE. Total RNA and protein lysates were collected for qPCR and western blot analysis for IgE receptor expression. Cell-free conditioned media were collected to quantify pro-inflammatory mediators by ELISA. IgE-binding to FcεRI was confirmed using a cell-based assay. IgE-induced FcεRI activation was detected by phosphorylation of downstream kinases.

RESULTS
Primary human AEC isolated from asthmatic and non-asthmatic lungs demonstrated significantly increased FcεRIα protein compared to non-asthmatic cells. mRNA expression of FcεRI β subunit and FcεRII (CD23b) subunits was significantly increased in asthmatic AEC. IgE binding to FcεRI on AEC was demonstrated in a cell based assay post-treatment with varying doses of IgE. IgE binding was inhibited in a dose dependent manner when AEC were pre-treated with the anti-FcεRI antibody that specifically targeted the IgE binding site. Activation of kinases downstream of FcεRI was observed in response to IgE treatment in primary non-asthmatic AEC.

CONCLUSION
Understanding of IgE receptor expression and function in airway epithelial cells will help us to further investigate the mechanism of allergic inflammation.

ABSTRACT #45

Evaluating the function of bronchial epithelial cells and their associated cytokine expressions of IL-17A and IL-17F

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BACKGROUND
Th17 cell produce IL-17, which is an important cytokine in inflammatory airway disease. Recent reports indicate that other cells are capable of producing IL-17 including B cells, Neutrophils NKT-cells [1]. IL-17 has six subtypes (from IL-17A to IL-17F), but there are structural similarities between IL-17A and IL-17F. Particularly, IL-17A and IL-17F bind to the same receptor subunits, IL-17 receptor A (IL-17RA) and IL-17RC heterodimer [2]. However, IL-17A and IL-17F have different roles in inflammation. IL-17A is associated with allergic inflammation, while IL-17F is associated mainly with infectious inflammation mainly. IL-17A and IL-17F are known to stimulate bronchial epithelial cells (BEC) to produce cytokines like IL-6, CXCL-1 and GM-CSF. In our study, we evaluated and compared the surface expression of IL-17RA and intracellular expressions of IL-17A, IL-17F, IL-6 and CXCL-1 in BEC between asthma, COPD and healthy subjects. In addition, we analyzed the relationship between these receptors and cytokines.

METHODS
We collected sputum cells from mild asthmatics (n=10), subjects of mild to moderate COPD (n=8), and healthy control (n=8). All asthmatics and COPD subjects had stable airway disease and were not on inhaled or oral corticosteroid therapy. We stained sputum cells with fluorescent labeled antibodies and relevant isotype controls, and evaluated the expressions of IL-17RA, IL-17A, IL-17F, IL-6 and CXCL-1 in BEC and CD4+ lymphocytes using flow cytometry (Becton Dickinson LSRII). We used expression of CD45- and CD326+ to detect BEC [3]. Data was analyzed using FlowJo software.

RESULTS
There were no significant differences in the expression of IL-17RA, IL-17A, IL-17F, IL-6 and CXCL-1 in BEC between asthmatic, COPD and healthy subjects. However, there was a positive correlation between surface expression of IL-17RA and intracellular expression of IL-17A, IL-17F and IL-6 in BEC in all subjects. In addition, we found a positive correlation between intracellular expression of IL-17A in CD4+ lymphocytes and surface expression of IL-17RA in BEC.

CONCLUSIONS
The current study did not find significant differences in the expression of cytokines and receptors associated with IL-17A and IL-17F in BEC. However, positive correlations between (i) IL-17A in CD4+ lymphocytes and IL-17RA in BEC and (ii) surface expression of IL-17RA and intracellular expressions of IL-17A, IL-17F and IL-6 in BEC suggest that IL-17A production by CD4+ lymphocytes may drive BEC production of IL-17A and IL-17F, through IL-17RA. Further investigation is required to fully understand the mechanistic relationship between CD4+ lymphocyte derived cytokines and activation of BEC.

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ABSTRACT #46

Resiquimod (S28463) treatment prevents the increase in airway resistance and decreases inflammation and serum IgE levels in *Ascaris suum* induced allergic asthma model in non-human primates

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BACKGROUND
Allergic asthma is a disease affecting the respiratory and immune systems. Peptides targeting toll like receptors (TLRs) have been explored as drugs for treating allergic asthma. In published studies using acute allergic asthma model in C57Bl/6 and A/J mice and chronic asthma model in Brown Norway rats, we demonstrated the therapeutic potential of TLR 7/8 ligand, resiquimod S28463 (S28) [1, 2]. Here, we validated our findings from the rodent models in a non-human primate (NHP, *Macaca fascicularis*) model of allergic asthma.

METHODS
We developed a protocol for establishing allergic asthma in non-allergic NHPs by sensitization and challenge to *Ascaris suum (A. suum)* antigen. NHPs were treated with S28 by nasogastric intubation 24 hours prior to antigen challenge. The potential of S28 as a treatment for allergic asthma was assessed by measuring airway responsiveness to methacholine exposure, serum IgE concentration, and by quantifying the concentration of inflammatory cytokines and cells in bronchoalveolar lavage fluid (BALF).

RESULTS
Sensitization followed by challenge with *A. suum* creates a successful allergic response in initially non-allergic NHPs. In allergic untreated animals, we observed an increase in serum IgE concentration, airway responsiveness, and inflammation in the BALF. Treatment with S28 prior to each allergen challenge caused a significant decrease in skin wheal area and total serum IgE concentration. S28 also prevented the increase in airway resistance observed with each subsequent allergen challenge. The level of inflammatory cells and cytokines in S28 treated and challenged animals were significantly different from challenged and untreated animals, and similar to those challenged with saline.

CONCLUSION
The data presented herein confirm that S28 has great potential as a therapeutic agent for allergic asthma. Studies on the molecular mechanisms responsible for this effect, as well as the potential when S28 is combined with other anti-asthma drugs remain to be explored.

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ABSTRACT #47

Novel blood-based biomarker panels of the late phase asthmatic response: from discovery to validation

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BACKGROUND
We have previously shown significant molecular changes in the blood between asthmatic individuals who elicit the dual response (dual responders, DRs) compared to those that develop the isolated early response (early responders, ERs) after allergen inhalation challenge [1, 2]. Identifying individuals likely to develop the dual response may aid in the screening of subjects for clinical trials which test drugs for the attenuation of the late phase asthmatic response or provide novel targets for therapeutics. The objective of this study was to develop blood-based biomarker panels that could identify asthmatic individuals with high probability of developing a late phase response.

METHODS
The discovery cohort consisted of 36 asthmatic individuals (15 ERs and 21 DRs) and an independent cohort of 45 (9 ERs and 36 DRs) asthmatic individuals made up the validation cohort. Blood samples were collected prior to allergen challenge. After RNA extraction, and globin depletion the total RNA was sequenced using an Illumina HiSeq 2000 as 100 bp paired end reads at Genome Québec. Both genome-guided datasets such as UCSC genes, UCSC gene-isoforms, and Ensembl and *de novo* assembled transcripts using the Trinity software [3] were constructed. Top ranked biomarker candidates were transferred to the clinically relevant nanoString platform and final biomarker panels were identified and locked down and validated in the external cohort.

RESULTS
Predictive biomarker panels had a classification performance (based on the area under the receiver operating curve, AUC) that ranged between 60-70% in the discovery cohort. 87 transcripts identified on the RNA-Seq platform were transferred to the nanoString elements platform. The transcripts were split with respect to their dataset of origin and validated in the external cohort. The UCSC gene-isoforms and Trinity biomarker panels had an AUC of 67% and 71%, respectively. The UCSC gene-isoforms panel was enriched with genes from the TCR Signaling pathway whereas the Trinity panel was enriched with Signaling by the B Cell Receptor (BCR) pathway.

CONCLUSIONS
Predictive gene biomarker panels in the blood are successful at identifying individuals likely to develop the late response upon allergen inhalation challenge. These biomarker panels consisted of novel transcripts that are representative of T cell and B cell biology. These panels will be evaluated in other allergic conditions such as allergic rhinitis where a similar phenomenon of the biphasic response exists.

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ABSTRACT #48

The Allergic Rhinitis Clinical Investigator Collaborative (AR-CIC) - Cytokine analysis of nasal secretions before and after nasal allergen challenges (NAC)

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BACKGROUND
The Allergic Rhinitis Clinical Investigator Collaborative (AR-CIC) has previously reported biological outcomes following changes to the protocol for nasal allergen challenge (NAC). Using our cumulative allergen concentration (CAC) protocol we investigated whether or not cytokine profiles were reproducible between two separate NAC visits (3 weeks apart).

METHODS
10 ragweed allergic participants were enrolled, all with AR symptoms in ragweed season and a supportive skin test response. During screening, 4-fold incremental concentrations of ragweed allergen were administered until each participant achieved the qualifying symptom score (TNSS >=8/12 and a 50% Peak Nasal Inspiratory Flow (PNIF) reduction). For the subsequent NAC1 visit, (21days later) participants were challenged with one dose of allergen equivalent to the cumulative amount of allergen received during screening. Nasal secretions were collected at baseline, 1hr, 6hr and 24hr post-NAC and cryopreserved. This procedure was repeated at a second NAC visit 21 days later (NAC2). Cytokine levels were determined using the Luminex®x-MAP[TM]Technology.

RESULTS
IL-5 and IL-13 were upregulated at 6hr and 24hr post NAC (Wilcoxon matched-pairs test NAC1: IL-5 $p=0.0652$ both time points, IL-13 $p=0.0273$ at 6hr; NAC2: IL-5 $p=0.0156$ at 24hr, IL-13 $p=0.0547$ at 6hr). IL-6 was upregulated at 1hr, MCP-1 and MIP-1b were upregulated at 24hr. Other pro-inflammatory cytokines, chemotaxis and growth factors remained mainly unchanged following NAC. In general, cytokine levels appeared slightly lower for NAC2.

CONCLUSIONS
Cytokine profiles appear very similar between both NAC visits using a small set of atopic samples. The 24hr time-point was an important addition to the AR-CIC protocol.

ABSTRACT #49

Window condensation as a moisture indicator in the Canadian Healthy Infant Longitudinal Development (CHILD) Study

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BACKGROUND
Adverse respiratory outcomes and the growth of detrimental microorganisms have been linked to excessive indoor moisture [1, 2]. Leaks are known to promote mould growth and increase indoor humidity, whereas the impact of houseplants on indoor moisture remains unclear. In this preliminary analysis, we explored the relationship between window condensation, mould, leaks, and houseplants in the CHILD Study 3-month home environment dataset [3].

MATERIALS/METHODS
CHILD Study's hypotheses, recruitment, inclusion and exclusion criteria, study sample demographics, methods, and clinical and environmental datasets are described elsewhere [3, 4].

For this analysis, we explored the relationship between moisture and mould, houseplants and leaks in the CHILD 3-month home environment dataset. The presence of window condensation was used as a proxy for indoor moisture. We applied a cumulative odds model, and controlled for the frequency of window openings in mid-winter and mid-summer, season (summer, fall, winter, spring), humidifying behaviours, and study center (Vancouver summer as the baseline).

RESULTS
In our adjusted model, mould (OR=3.39; 95%CI: 2.98-3.86) and leaks (OR=1.25; 95%CI: 1.044-1.48) were more likely to be present in homes with window condensation. There was no significant association between houseplants and window condensation.

CONCLUSIONS
Window condensation is correlated with leak and mould occurrence, but not with the presence of houseplants.

ACKNOWLEDGEMENT

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ABSTRACT #50

Prenatal smoke exposure alters mitochondrial DNA methylation in umbilical cord blood dendritic cells

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BACKGROUND
Epigenetic alterations, including changes in DNA methylation, are related to various perinatal environmental factors that affect child health, such as maternal smoking, C-section, gestational weight gain and maternal allergy [1-7]. Human cells contain nuclear and mitochondrial DNA (mtDNA), but the impact of mitochondrial epigenetics on the development of environmentally-linked diseases, such as allergies and asthma, has not been adequately explored. Mitochondria are particularly sensitive to oxidative stress, responding with changes in copy number and mtDNA methylation [8; 9]. Dendritic cells were targeted because of their sensitivity to environmental stimuli and importance in allergic disease [10-13]. We tested the hypothesis that prenatal smoke exposure, a source of oxidative stress during fetal development, would be associated with mitochondrial epigenetic differences in dendritic cells at birth.

METHODS
Umbilical cord blood dendritic cells were isolated using magnetic sorting (n=91). Samples were drawn from a cohort with a known high prevalence of maternal smoking, the Kingston Allergy Birth Cohort [14]. We analyzed mtDNA regions with potential functional impacts, including the D-loop promoter, transfer RNA phenylalanine (MTTF), and 12S ribosomal RNA (MT-RNR1) by pyrosequencing [8]. Copy number was determined using qPCR. Copy number and mtDNA were analyzed for associations with perinatal factors using models adjusted for maternal age, pre-pregnancy BMI, ethnicity, child's gender, and SES.

RESULTS
The subset of the cohort with umbilical cord blood available for mtDNA analyses did not exhibit significant differences from the Kingston Allergy Birth Cohort as a whole in terms of maternal age, pre-pregnancy BMI, weight gain during pregnancy, sex of the child, mode of delivery, siblings, maternal allergy, income, gestational age or maternal exposure to cigarette smoke. Prenatal smoke exposure was associated with a 1.53% (95% CI: 0.61 - 2.46%, p=0.002, adjusted model) increase in mtDNA methylation in MTTF. The D-loop mtDNA region in dendritic cell mitochondria also demonstrated an increase in methylation associated with maternal smoke exposure during pregnancy, of 3.82% (95% CI: 0.50 - 7.14%, p=0.03, adjusted model). However, we did not observe significant associations between mtDNA methylation and gestational weight gain, maternal allergy or C-section delivery. MtDNA copy number was also not associated with any of the perinatal risk factors examined.

CONCLUSIONS
Maternal smoking was associated with differences in umbilical cord blood dendritic cell mitochondrial DNA methylation in 2 out of the 3 regions examined. These effects on mtDNA may be related to the known effects of smoking on oxidative stress balance, and may affect dendritic cell function and the development of allergic disease.

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ABSTRACT #51

The effects of perinatal distress, immune biomarkers and mother-infant interaction quality on childhood atopic dermatitis (rash) at 18 months

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BACKGROUND
Perinatal psychosocial distress, including stressful life events, anxiety and depression are risk factors for childhood atopic dermatitis (rash) [1]. Maternal psychosocial distress is associated with excess prenatal stress hormones [2] and reduction in the quality of maternal-infant interaction [3], which may affect biomarkers of infant immunity such as interleukin (IL) levels and predispose the growing child to rash [4, 5]. This study will: (1) describe the association between perinatal distress and infant interleukins at 3 months of age, (2) describe the association of interleukins and atopic dermatitis (rash) and (3) build a best fit model from the identified associations in (1) and (2).

METHODS
120 women reported distress levels during pregnancy and at 3 months postpartum. Venous blood was collected from their 3 month old infants to assess plasma interleukin levels. Maternal-child interaction was measured 6 months postpartum with the Nursing Child Assessment Teaching Scale. Presence and number of skin areas affected by rash were assessed via parent report at 18 months. Correlation and multiple regression analyses identified the best fit model for rash using forward stepwise regression.

RESULTS
Prenatal depression ($r=-0.23$, $p=0.01$) and stressful life events pre- and postnatally ($r=0.27$, $p=0.00$) were associated with IL2p70. Pre- and postnatal anxiety were associated with IL8 ($r=-0.28$, $p=0.02$) and IL4 ($r=-0.24$, $p=0.04$). IL10 was associated with child skin rash ($r=-0.25$, $p=0.01$) at 18 months. 20% of the variance in childhood rash at 18 months was explained by the model (Table 1).

Table 1: Multiple Regression Analysis for Variables Predicting Child Skin Rash

Variables	Estimate	Standard Error	P-value
Maternal Age	-0.03	0.01	0.023
Maternal Education	0.11	0.06	0.091
IL10	-1.31	0.41	0.002
Perinatal Stress	-0.12	0.06	0.057
Maternal-Infant Interaction Quality	0.02	0.01	0.018

Note: IL10 was transformed by taking the square. * P <0.05

CONCLUSIONS
Perinatal distress is associated with elevated infant ILs. Demographic variables (maternal age, education), IL10, perinatal stressful life events (*e.g.* separation/divorce, family death) and maternal-infant interaction best predicted skin rash in 18 month old infants. We will now evaluate how maternal-child interaction moderates associations between both (1) perinatal distress and (2) immune biomarkers, and atopic dermatitis (rash) in children at 18 months.

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2016 ALLERGEN POSTER COMPETITION

KNOWLEDGE TRANSLATION CATEGORY

ABSTRACT #52 - KT

A microorganism found in high density in air of agricultural environments induces a hypersensitivity lung response

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PURPOSE
Bioaerosols in environments are associated with the development of inflammatory lung diseases [1]. The microorganism *Methanosphaera stadtmanae* (MSS) is found in high concentrations in agricultural environments bioaerosols [2-4] and MSS induces lung inflammation in mice [5]. However, whether this response is likely to induce pathology in humans is not known.

FINDINGS
MSS induces an inflammatory response involving cellular mechanisms that could be potentially harmful for human pulmonary health if they are chronically exposed to this new allergen.

POTENTIAL USE
Assessment of risk to develop chronic airway inflammatory diseases in agricultural environments is inefficient as not all causal agents are identified. Identification of MSS as a potential inducer of these diseases could therefore help to assess the risk of developing such diseases.

IMPORTANCE OF RESEARCH
Chronic airway inflammatory diseases have disastrous effects on workers, especially when the cause is not identified, as removal from the source of the disease, therefore unemployment, are the only effective treatments. Quantification of MSS in the air could prevent this outcome by allowing to take measures (such as better ventilation system, wearing a particulate safety mask, *etc.*) to avoid the potential harmful effects of chronic exposure.

ACKNOWLEDGEMENTS

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ABSTRACT #53 - KT

Are children who were exposed to parental smoking as babies more likely to experience allergic symptoms after eating certain foods?

Laura Y. Feldman[1,2*], Jesse D. Thacher[3,4], Marianne van Hage[5,6], Inger Kull[3,4,7], Erik Melén[3,4,7], Göran Pershagen[3,4], Magnus Wickman[3,7], Teresa To[1,2], Jennifer LP Protudjer[3,4]†, Anna Bergström[3]†

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PURPOSE
The blood of children whose parents smoked around them when they were babies contains higher levels of proteins that are involved in allergic reactions to foods [1]. The purpose of this study was to determine whether children who were exposed to parental smoking when they were babies are more likely to experience allergic symptoms after eating certain foods than children who were not exposed.

FINDINGS
In a large group of Swedish children who have been studied from infancy to adolescence, we found that children whose parents smoked around them when they were babies were more likely to experience symptoms when they ate certain foods and to have higher levels of proteins in their blood that are involved in allergic reactions to foods. This relationship was partly because of smoke-exposed children reacting to peanuts more often than non-exposed children.

POTENTIAL USE
Our findings support the actions of policymakers and individuals to reduce children's exposure to secondhand smoke in the months following birth.

IMPORTANCE OF RESEARCH
There are few established health behaviours that are known to increase children's risk of experiencing symptoms to foods. Our research points to a health behaviour that could potentially be intervened in to reduce this risk.

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ABSTRACT #54 - KT

Integrating complex data to understand the causal factors of allergy and asthma: a pilot study

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PURPOSE

The Canadian Healthy Infant Longitudinal Development (CHILD) Study utilizes biological sampling, home assessment and questionnaires to understand the effects of genetic predisposition and environmental factors on child health and development. Questionnaire data is currently housed in HealthDiary, a portal which stores questionnaires and responses as spreadsheets of unhomogenized values which are difficult to integrate. With a robust database schema, this rich data could be more fully leveraged.

FINDINGS

We are implementing an ontological framework to create a pilot database integrating skin allergy testing and early childhood wheeze data which represent two of the most frequently queried and priority areas.

POTENTIAL USE

Through defining variables and criteria, and mapping these fields to community-accepted standards, we are creating a flexible database schema and interface which will enable integration of other types of information such as genetic and microbiome data, chemical and environmental exposure, nutrition and socioeconomic factors.

IMPORTANCE OF RESEARCH

We are working towards integrating various data types from the CHILD Study in a single database allowing researchers to carry out complex queries and better leverage research which will provide key insights into the development of allergy and asthma.

ACKNOWLEDGEMENTS

ABSTRACT #55 - KT

The preclinical journey of resiquimod (R-848) – a promising treatment for allergic asthma

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PURPOSE
New drugs for asthma management are high in demand, especially for patients whose disease is uncontrolled by existing medications. We explored if a compound known as R-848 can offer protection against allergic asthma. Before testing the potential of this drug in patients, we verified if any benefit can be observed in three different animal models of allergic asthma (mouse, rat and monkey).

FINDINGS
Our findings show that giving a single dose of R-848 before allergen exposure can prevent immune, respiratory, and lung symptoms in all three animal models. The protection offered by this drug is not specific to one type of allergen; in fact we tested egg-derived and parasite-derived allergens in our animal models.

POTENTIAL USE
Our study proposes R-848 as a novel treatment for allergic asthma that deserves further study. After understanding how this drug works, it could either be combined with existing treatments or new drugs that offer better asthma control could be designed.

IMPORTANCE OF RESEARCH
It is important that new treatments are discovered for this disease because existing treatments do not help all asthmatics. The strength of our research is highlighted by our exploration of the benefits of R-848 in three different animal models using different types of allergens.

ACKNOWLEDGEMENT

ABSTRACT #56 - KT

What is happening inside of asthmatics lungs? New imaging technique can help us to answer it

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PURPOSE
The normal lung is able to inflate and deflate as we breathe due to elastic fibres. However, in asthma the airways within the lung are continually injured and the build-up of scaring due to non-elastic fibres such as collagen reduces the normal lung capacity. We are using a new imaging technique, called nonlinear optical (NLO) microscopy for the first time on asthmatic and non-asthmatic lungs to understand the alterations in the lung tissues with disease.

FINDINGS
Changes such as fiber length, thickness, and alignment can be quantified by examining NLO images. NLO images show collagen fibers in asthmatic lungs are less organized compared to the non-asthmatics lungs.

POTENTIAL USE
The ultimate goal is to provide researchers with important information to develop medications to repair collagen/elastin fibers to prevent scaring of the lung.

IMPORTANCE OF RESEARCH
The number of Canadian's that will die from asthma each year is currently estimated at 200 per annum. Asthma treatments are the second most expensive disease to treat and scaring changes within the asthmatic lung are currently unresponsive to existing asthma medications. By using NLO microscopy we are able to look how the lung structure is changing with disease.

ACKNOWLEDGEMENTS

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ABSTRACT #57 - KT

Impacts of environmental exposures on incidence and trajectories of childhood asthma

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PURPOSE
Given the heterogeneity of asthma and the diverging results on the impact of environmental exposures, we followed over 65,000 children from birth until age 10 for whom air pollution and greenness measures were derived.

FINDINGS
Surrounding greenness had a modest protective effect, independent of the impact of traffic pollution exposure during pregnancy, which increased the risk of developing asthma during the first 5 years of life. Children whose mothers lived close to highways during pregnancy had a 25% increased risk. The majority of new asthma cases were persistent throughout follow up.

POTENTIAL USE
Pregnant mothers' exposure to traffic pollution is likely a risk factor for asthma development, especially among children who developed this chronic condition in early infancy. Measures such as zoning and residential development away from major roads can help prevent damaging lung conditions. Patient management could also highlight individual protective measures including high-efficiency particulate air filters in the home, and the online use of air quality health indices.

IMPORTANCE OF RESEARCH
Air pollution affects 100% of the population and here we highlight the harmful effects of air pollution right from the moment we are created, even in urban areas with relatively low levels of pollution.

ABSTRACT #58 - KT

Peptide immunotherapy for treatment of peanut allergy

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PURPOSE
The prevalence of peanut allergy has doubled in the past 10 years, and currently stands at approximately 2% [1]. Despite its clinical severity and rising prevalence, there is a marked absence of widespread, practical treatments available for peanut-allergic patients. Peptide immunotherapy, a disease-modifying treatment that uses short peptides recognized by T cells, has been shown to reduce symptoms of allergic rhinoconjunctivitis. We have evaluated the ability of peptide immunotherapy to protect against anaphylaxis in a mouse model of peanut allergy.

FINDINGS
Mice treated with peptides from the major peanut allergen Ara h 1 were significantly protected from severe anaphylactic reactions to peanut, as measured by: clinical signs of allergic reaction, drop in core body temperature, and vascular leakage. They exhibited lower levels of peanut-specific allergic antibodies and decreased recruitment of inflammatory cells to the site of allergen challenge.

POTENTIAL USE/IMPORTANCE
Successful demonstration and characterization of peanut-specific peptide immunotherapy in this model will not only contribute to our knowledge of the immunological mechanisms at work in allergy and immune tolerance, but also pave the way for the creation of a human immunotherapeutic, which would prove life changing for peanut-allergic patients and their families.

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EVALUATIVE CRITERIA

Posters will be judged on:

Research Category

- degree of scientific rigour and originality of the research
- visual quality of the poster
- ability to communicate research effectively and respond to questions
- quality of plan to disseminate research findings to knowledge users

Knowledge Translation Category

- visual quality of the poster
- ability to communicate the purpose, findings, potential use and importance of the research in a manner that is understandable and captivating to a non-scientific audience
- ability to respond to questions effectively
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Prizes will be awarded for the top posters in each category. The number of prizes awarded in each category varies based on the number of abstracts submitted.

Thank you to the panel of judges:

Barbara Ally	Terry Delovitch	Michael Kobor
Meghan Azad	Delbert Dorscheid	Anita Kozyrskyj
Darryl Adamko	Sylviane Duval	Oxana Latycheva
Dean Befus	Susan Elliott	Kelly McNagny
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Louis-Philippe Boulet	Gail Gauvreau	Danuta Radzioch
Michael Brauer	John Gordon	Padmaja Subbarao
Deborah Danoff	Laurie Harada	Stuart Turvey

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ABSTRACT #21

What do MRI ventilation defects reveal about asthma control?

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BACKGROUND
Improving asthma control is a primary treatment goal for patients with severe asthma. Recent studies using multiple-breath-gas-washout techniques have suggested that poor asthma control may be related to ventilation heterogeneity [1, 2]. We previously determined the relationship between magnetic resonance imaging (MRI) ventilation abnormalities and asthma severity, but the links between asthma control and ventilation defects have not been ascertained. Thus, here our objective was to determine the relationship between ventilation defects, visualized using MRI, and asthma control. We hypothesized that MRI ventilation defects would be related to biomarkers of asthma control.
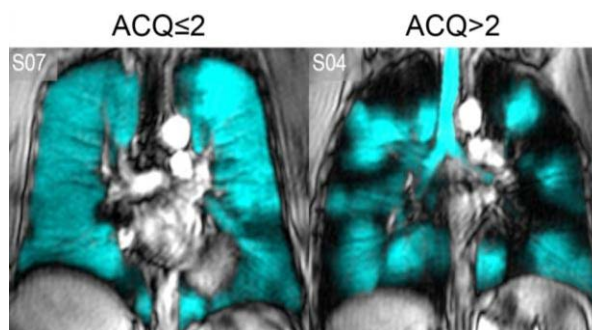
METHODS
Volunteers with severe asthma provided written-informed-consent to an ethics-board-approved protocol and underwent spirometry, multiple-breath-nitrogen-washout (MBNW) and noble gas MRI during a single two-hour visit. Asthma control and quality of life were evaluated using the Asthma-Control-Questionnaire (ACQ) and Asthma-Quality-of-Life-Questionnaire (AQLQ). Ventilation heterogeneity was quantified using the lung-clearance-index (LCI) and MRI ventilation-defect-percent (VDP) [3]. Univariate relationships were evaluated using Pearson correlations (r). Unpaired t-tests were performed to compare ventilation heterogeneity in subjects stratified by ACQ (≤ 2 or >2) and AQLQ scores (≥ 5 or <5) and previous 6-month exacerbation history (<1 or ≥ 1). All statistical analyses were performed using GraphPad Prism version 6.02 (GraphPad Software Inc.; La Jolla, CA, USA).

RESULTS
We evaluated 18 severe asthmatics (46±12 years) with poorly-controlled disease (ACQ=2.3±0.9). Mean VDP was 12±11% and LCI was 10.5±3.0. Both VDP (p=0.008) and LCI (p=0.02) improved post-bronchodilator. Whereas VDP was strongly correlated with LCI (r=0.86, p<0.0001), the post-bronchodilator change in VDP and LCI was not correlated (p=0.08). As shown qualitatively in Figure 1, there was a greater number and volume of MRI ventilation abnormalities in participants with worse ACQ. There was also significantly worse VDP but not LCI in asthmatics with ACQ >2 (p=0.04) and AQLQ<5 (p=0.04). There was a trend towards worse VDP (p=0.053), but not LCI (p=0.3), in asthmatics reporting ≥ 1 exacerbation in the past 6-months. VDP (but not LCI, p=0.052) was correlated with ACQ (r=0.62, p=0.01) but not AQLQ or exacerbation history.

Figure 1. Hyperpolarized ^{3}He MRI of two representative patients with severe asthma. Centre coronal slice ^{3}He MRI ventilation in blue co-registered to anatomical ^{1}H MRI in grey-scale for two representative severe asthmatics. S07 ACQ$\leq$2: 55 year-old female, FEV_1=77%$_{pred}$, ACQ=1.4, LCI=9.7, VDP=2%; S04 ACQ>2: 60 year-old female, FEV_1=47%$_{pred}$, ACQ=4.3, LCI=14.0, VDP=35%.

CONCLUSIONS
MRI ventilation defects were significantly worse in patients with worse asthma control and quality of life. This is important because regional MRI ventilation defects may be considered as a sensitive intermediate endpoint of asthma control. Such ventilation abnormalities can be rapidly and serially quantified to help guide treatment in order to improve asthma control.

2016 ALLERGEN POSTER COMPETITION

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