AllerGen

Allergy, Genes and Environment Network

Le réseau des allergies, des gènes et de l'environnement

TRAINEE POSTER ABSTRACTS

Seventh Annual Research Conference:
Innovation from cell to society[7]

Royal York Hotel, Toronto, Ontario
February 5-7, 2012

Networks of Centres of Excellence
Réseaux de centres d'excellence

About AllerGen

AllerGen NCE Inc., the Allergy, Genes and Environment Network, (est. 2004), is a national research network funded by Industry Canada through the Networks of Centres of Excellence (NCE) Program. AllerGen's mandate is to support research, networking, commercialization, knowledge mobilization and capacity building activities that contribute to reducing the morbidity, mortality and socio-economic impacts of allergic diseases.

AllerGen's long-term goal is to create an enduring network of allergy and immune disease experts whose discovery and development efforts contribute to reducing the impact of allergic and related immune diseases nationally and globally.

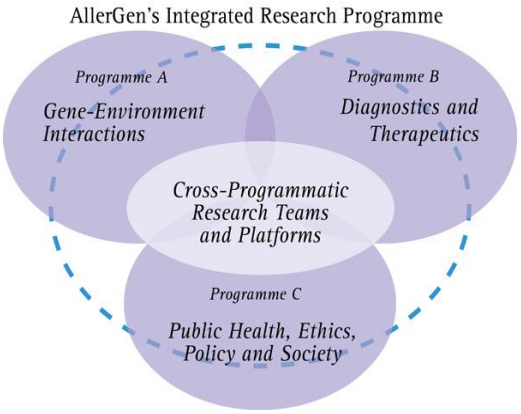
AllerGen invests in leading-edge, multidisciplinary allergy, asthma and anaphylaxis research of strategic importance to the generation of new knowledge that contributes to improved health and productivity of Canadians and benefits the Canadian economy.

AllerGen's research investments are directed towards three overarching themes:

1. ***Programme A – Gene-Environment Interactions***
   Strategic Focus: Genetics, environmental exposures and gene-environment interactions in allergy and asthma

2. ***Programme B – Diagnostics and Therapeutics***
   Strategic Focus: Biomarkers, immune monitoring and drug development/discovery

3. ***Programme C – Public Health, Ethics, Policy and Society***
   Strategic Focus: Allergic disease management and surveillance



Within these three themes, AllerGen also invests in four cross-programmatic, multidisciplinary research teams:

Established Cross-programmatic Teams

- The Canadian Healthy Infant Longitudinal Development (CHILD) Study
- Food Allergy and Anaphylaxis

Emerging Cross-programmatic Teams

- Mind-Body Interactions and Allergic Disease
- Occupational and Work-related Allergy and Asthma.

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3A	**McLean,** Kathleen	MSc	Simon Fraser University	Tim Takaro	An Exploratory Approach to 'Total Exposure Assessment' in the CHILD Study: Preliminary Validation of an Environment Exposure Index
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11A	**Hsu,** Karolynn	MSc	University of British Columbia	Stuart Turvey	Exploring the Functional Role of *ORMDL3* in Innate Immunity
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13A	**Sava,** Francesco	MD, MSc	University of British Columbia	Christopher Carlsten	Diesel Exhaust and Neurogenic Airway Inflammation

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15A	**Alton**, Megan E.	UG	McGill University	Anita L. Kozyrskyj	Postpartum Depression: An Independent Predictor of Wheeze in Preschool Girls
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20A	**Azad,** Meghan	PDF	University of Alberta	Anita L. Kozyrskyj	Exclusive Breastfeeding Protects Against *Clostridium difficile* Colonization by Promoting Lower Relative Abundance of Lachnospiraceae in Gut Microbiota: Implications for Atopic Disease?
21A	**Sallehy,** Sina	UG	McMaster University	Judah A. Denburg	Effect of Thymic Stromal Lymphopoietin (TSLP), Interleukn (IL)-25 and IL-33 on Cord and Peripheral Blood CD34+ Progenitor Cell (HPC) Differentiation
22A	**Simons,** Elinor	PhD	The Hospital for Sick Children	Teresa To Sharon Dell	Is Breastfeeding Protective against the Development of Asthma or Wheezing in Children? A Systematic Review and Meta-Analysis

1A: Indoor Measurements and Multimedia Modeling of Phthalates: Toronto Intensive (TI) Homes

Programme A: Gene-Environment Interactions

Authors: **Sri R. Chaudhuri**, Evelyn Mukwedya, Stephanie Verkoeyen, Miriam L. Diamond, University of Toronto
Supervisor: Dr. Miriam L. Diamond

Objectives/Purpose: Phthalates esters (PAE) represent an ubiquitous class of plasticizers. They are found in a variety of consumer products, such as personal care items, residential materials, and medical applications. Several studies have indicated an association between PAE exposure and asthma and allergy development in children. The objective of the current study is to: (i) determine optimal indoor sampling methods of PAE and other semi-volatile organic compounds (SVOCs) that relate to exposure and (ii) develop a mechanistic multimedia model capable of estimating indoor chemical concentrations and fate.

Methods: Intensive sampling campaigns were executed in five Toronto homes over four days in September 2010 and August 2011. Measurements were obtained for multiple rooms within homes and organized into: [1] building characterization parameters (such as i) air-exchange-rate (AER), ii) temperature, iii) particulate matter (PM) concentration, and iv) dwelling dimensions), [2] household concentrations of six PAE and other SVOCs in dust, indoor air, and surface films (collected using both standard and novel techniques), and [3] participant specific exposure measures including daily morning urine samples to monitor PAE metabolites. During the 2011 TI, daily dermal wipes of participant's hands and forehead, as well as dust samples from participant's offices were collected. Indoor dust samples were also obtained at each residential site as per CHILD protocol for comparison between the dust sampling methods.

Findings: Over 500 samples and 190 hours of continuous measurements have been collected over the course of the two TI sampling campaigns. Average temperature and $PM_{2.5um}$ concentrations in the homes roughly ranged between 20 ^{o}C to 30^{o}C, and 3 ug/m^{3} to 30 ug/m^{3}, respectively. Average PAE window film concentrations between homes ranged from 10^{-3} to 3 ng/cm^{2} of surface. In 100% of the homes heavier molecular weight di(2-ethylhexyl) phthalate (DEHP) and diisononyl phthalate (DiNP) had the highest concentrations in window films, whereas lighter molecular weight diethyl phthalate (DEP) was found to be the lowest. The average concentration of different urinary PAE metabolites among the participants ranged between a few ng/mL, up to 150 ng/mL. In addition to daily morning voids, multiple urine samples collected over the span of a 12 hour period varied up to two orders of magnitude in a single participant for some PAE concentrations. Further, PM and PAE urinary metabolite concentration data will also be discussed.

Deliverables: The TI study will provide an evaluation of sampling techniques aimed at assessing exposure. Furthermore, the use of passive samplers to measure indoor air PAE concentrations is a new application, and will serve as a benchmark for future studies. The multimedia indoor model is being designed to effectively explain phthalate concentrations and offer a cost-effective assessment tool.

Relevance: The current research will improve the ability to identify sources, and explain fate, transport, and exposure of PAE and other SVOCs in the indoor environment. This will allow for the suggestion of interventions or preventative measures to minimize exposure to these chemicals and their adverse effects. The insight gained will also permit effective use of exposure data obtained from CHILD and with the potential to lead to straightforward and cost-effective exposure measurement techniques that may be used in future time points in CHILD.

2A: Is House Dust a Reservoir for Gut Bacteria?

Programme A: Gene-Environment Interactions

Authors: **Tedd Konya**[1], B. Koster[1], H. Maughan[1], J. Ewaze[1], M. Azad[2], D. Guttman[1], A.B. Becker[3], A. Kozyrskyj[2], J. Scott[1]
[1] University of Toronto [2] University of Alberta [3] University of Manitoba
Supervisor: Dr. James Scott

Objective/Purpose: Numerous studies have identified an association between the infant gut microbiota and the development of asthma and atopic disease. Certain environmental exposures are known to shape the succession of infant gut microbiota; including birth delivery mode, antibiotic use, breast or formula feeding, and the mother's own microbiota. This study investigates the extent to which the indoor environment itself may serve as a reservoir for gut bacteria. This study compares the microbiota of household dust with that of infant fecal samples collected at three months to compare microbial community composition.

Methods: Samples of infant stool and household dust were obtained for twenty, three month-old infants from Winnipeg, Canada; these subjects were early recruits in the Canadian Healthy Infant Longitudinal Development (CHILD) study. For each sample, community bacterial 16S rDNA was sequenced using a novel Serial Illumina Sequencing (SI-seq) method. Endotoxin levels in dust were analyzed by *Limulus* Amoebocyte Assay. Summary and statistical analyses were performed using MS Excel, PC-ORD, and MiniTab 16.

Findings: Both stool and dust samples were dominated by members of the phyla Actinobacteria, Firmicutes, and Proteobacteria, with dust samples also having a large content of Cyanobacteria. Analysis by nonmetric dimensional scaling (NMDS) revealed dust and stool communities as two distinct groups, with stool further divided into two subgroups. Despite these distinct community structures, several genera were present in both stool and dust. The genera most commonly observed in both samples for a given subject were: *Streptococcus*, *Bifidobacterium*, *Veillonella*, *Actinomyces*, and *Clostridium*. In dust samples, endotoxin levels were statistically correlated with the proportion of gram-negative bacterial 16S signatures ($p=0.01$, $r= 0.572$). However, no correlation was found between gram-negative bacteria in stool and either dust endotoxin ($p= 0.63$) or the proportion of gram-negative bacteria in dust ($p= 0.17$).

Deliverables: The findings demonstrate a clear difference in the microbiomes of infant gut and household dust. However, certain taxa co-occur frequently in both dust and stool, suggesting bacteria may move from dust to the gut, or *vice versa*. Additionally, while endotoxin levels were correlated with gram-negative bacterial proportions in dust, there does not appear to be an association between dust and stool when looking at endotoxin levels or proportion of gram-negative bacteria.

Relevance: These results show that the way in which the indoor environment shapes the infant gut microbiota deserves a detailed investigation. Results from such studies could ultimately serve to help to reduce asthma and atopic disease by guiding modifications of the indoor environment for the promotion of early life gut colonization by beneficial microbes.

3A: An Exploratory Approach to 'Total Exposure Assessment' in the CHILD Study: Preliminary Validation of an Environmental Exposure Index

Programme A: Gene-Environment Interactions

Authors: **Kathleen McLean**[1], Jeff Brook[2], James Scott[3], Malcolm Sears[4], Ryan Allen[1], Michael Brauer[5], Huan Shu[1], Padmaja Subbarao[3], Stuart Turvey[5], Allan Becker[6], Piush Mandhane[7], Tim Takaro[1].
[1]Simon Fraser University; [2]Environment Canada; [3]University of Toronto; [4]McMaster University; [5]University of British Columbia; [6]University of Manitoba; [7]University of Alberta
Supervisor: Dr. Tim Takaro

Objective/Purpose: The Canadian Healthy Infant Longitudinal Development (CHILD) Study aims to determine the effects of a range of environmental and genetic factors which may impact the development of allergy and asthma in children. Environmental exposures in the home environment are being assessed through multiple questionnaires, a home inspection and analysis of house dust and biological samples, yielding a large and rich dataset. A major challenge is determining how to use these data to fully characterize all environmental exposures of interest and to understand their possible relationships to outcomes of interest. As an exploratory approach, a preliminary 'Environmental Exposure Index' has been developed based on the physical home environment when the infant is three months of age. As a preliminary validation of the index, this poster looks at whether the index predicts exposure to phthalates, using urinary phthalate metabolite concentrations as biomarkers of exposure. Phthalates are a common household chemical thought to modulate inflammation. The poster aims to stimulate discussion about exposure assessment and modeling for the CHILD Study.

Methods: The Environmental Exposure Index was calculated for 501 CHILD subjects using data from the home inspection, as well as questionnaires completed at three months. Points are given for the presence of potential inflammatory exposures in the home environment and summed to generate the index score. The selection of exposures and their weighting was informed by a review of relevant literature. Exposures are grouped into 15 domains including: floors, walls and furniture; cleaning and chemical products; heating and cooking; baby care products; dust; moisture; mould; environmental tobacco smoke; and pets. Simple linear regression was used to find associations between the exposure domains and phthalate metabolite concentrations from infant urine samples collected at three months of age.

Findings: Higher levels of phthalate metabolites in urine were associated with higher index scores in the following exposure domains: floors, walls and furniture; mould; pools; water leaks; environmental tobacco smoke; and general environment. Levels of mono-2-ethyl-5-hydroxyhexyl phthalate as well as the sum of metabolites of di-(2-ethylhexyl) phthalate were positively associated with the sum of the index exposure domains. These findings are similar to results from a multiple linear regression analysis using the same data, but with a different approach to using the questionnaire and home assessment data.

Deliverables: First iteration of a comprehensive exposure index for the CHILD Study.

Relevance: This work-in-progress contributes to on-going efforts to understand the combined contributions of the diverse and complex exposure data being collected through the CHILD Study. Subsequent iterations of the index will include other exposures related to medications, diet and stress, and will explore associations with clinical endpoints potentially modulated by inflammation, such as intrauterine growth, airway inflammation and immune markers.

4A: Effect of Diesel Exhaust on the Airway Response to Allergen

Programme A: Gene-Environment Interactions

Authors: **Mandy Pui**[1], Meaghan MacNutt[1], Thomas Sandstrom[2], Anders Blomberg[2], Chris Carlsten[1]
[1]University of British Columbia, Vancouver, Canada, [2]Umeå University, Umeå, Sweden
Supervisor: Dr. Christopher Carlsten

Objective/Purpose: Asthma symptoms are known to be dependent on both allergenic and irritant exposures. The mechanisms that underlie the association between traffic-related air pollution and asthma remain unclear. Specifically, there is limited evidence in animal models and human nasal models that diesel exhaust (DE) augments the response to allergen, but this has not been shown *in vivo* in the human lung. Bronchial epithelial cells were initially considered only as a physical barrier, but have been shown *in vitro* to secrete CCL20 and be involved in recruiting adaptive immune cells. Our objectives are to determine whether DE augments the allergen (Th2) response *in vivo* in the human lung, such that the effects are supra-additive, and whether or not bronchial epithelial cells play a role in this augmentation.

Methods: We recruit volunteers and determine their specific sensitization by skin prick testing. Each subject participates in a blinded crossover experiment between two conditions (filtered air (FA) and 300μg $PM_{2.5}/m^3$ of DE), randomized and counter-balanced to order. Each subject is exposed to each condition, with a 4-week washout period between exposures. One hour following exposure, diluent-controlled segmental allergen challenge is performed using 5mL of saline and allergen extract (in a concentration 10-fold lower than the lowest concentration, producing a positive (≥3mm) wheal) in the lingular and right middle lobe segments. Two days post-exposure initiation, bronchial washings (BW) and bronchoalveolar lavages (BAL) are collected in both the allergen-affected and control regions. The levels of Th1 (IFN-gamma, IL-2, IL-12p70) and Th2 (IL-4, IL-5, IL-10, IL-13) cytokines as well as CCL20 are evaluated in BW and BAL using commercial ELISA kits. DE is determined to have a supra-additive effect on the response to allergen if the average cytokine level shows this pattern: (DE + Allergen) **>** [(DE + Saline) + (FA + Allergen)].

Findings: Three subjects have completed the study to date. On average, DE had a supra-additive effect on Th2 cytokines IL-5 and IL-13 in both the BW and BAL [mean concentrations for (DE + Allergen), (DE + Saline), (FA + Allergen) respectively, of IL-5 in BW = 18, 0.5, 8 pg/mL; IL-5 in BAL = 63, 2, 30 pg/mL; IL-13 in BW = 4, 0.4, 0.8 pg/mL; IL-13 in BAL = 6, 0.5, 2 pg/mL). DE also had a supra-additive effect on CCL20 in the BW [mean concentrations for (DE + Allergen), (DE + Saline), (FA + Allergen) = 123, 39, 52 pg/mL, respectively]. There were no apparent supra-additive effects observed in the other cytokines tested.

Deliverables: Preliminary results suggest that DE has a supra-additive effect on the airway response to allergen in previously sensitized individuals. Bronchial epithelial cells may play a role in this augmentation. We expect a sample size of 18 subjects by the end of 2012.

Relevance: Efforts to understand mechanisms of health effects due to traffic-related air pollution, in order to develop more accurate public policies to protect vulnerable populations, are dependent on a high-quality model. This is the first study to determine whether DE augments the airway response to allergen *in vivo* in the human lung, such that the effects are supra-additive. Findings from this study will be communicated to decision-makers via international conferences and peer-reviewed journals and directly to regulators such as WorkSafeBC.

5A: Potential Sources of Phthalate Exposure in the CHILD Study at Three Months of Age

Programme A: Gene-Environment Interactions

Authors: **Huan Shu**, BA[1], Leilei Zeng, PhD[1], Ryan Allen, PhD[1], Malcolm Sears, MB, ChB, FRCPC, FAAAAI,[2] Jeff Brook, PhD[3], James Scott, PhD[4], Mike Brauer, ScD[5], PJ Subbarao, MD, MSc, FRCP[8], Allan Becker, MD[6], Piush Mandhane, MD[7], Stuart Turvey, MB BS DPhil FRCPC[5], T Takaro, MD, MPH, MS[1]
Simon Fraser University, Burnaby, B.C. Canada[1], McMaster University, Hamilton, ON[2], Environment Canada, Toronto, ON[3], University of Toronto, Toronto, ON[4], University of British Columbia, Vancouver, B.C. Canada[5], University of Manitoba, Winnipeg, MB, Canada[6], Strollery Children's Hospital, Edmonton, AB[7], The Hospital for Sick Children, Toronto, ON[8]
Supervisor: Dr. Tim Takaro

Objective/Purpose: The Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort study is probing determinants of asthma and allergy in early life. Exposure to the ubiquitous plasticizers known as phthalates may contribute to the development of an inflammatory response and be a factor in the development of allergic disease through direct or adjuvant mechanisms.

Methods: We have analyzed phthalate metabolites (monobutyl phthalate (MBP); monobenzyl phthalate (MBzP); mono-ethyl phthalate (MEP); mono-2-ethyl-5-oxohexyl phthalate (MEOHP); mono-2-ethylhexyl phthalate (MEHP); mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP) in urine samples from 365 subjects at age three months. Subjects were recruited from four major cities in Canada: Vancouver, Edmonton, Winnipeg, and Toronto. Multiple regression along with geographic analyses were used to examine associations between levels of urinary phthalate metabolites and measurements of phthalates in house dust and questionnaire/home inspection indicators of phthalate sources in the indoor environment, including furnishings and personal care products.

Findings: We found detectable levels of metabolites MEP, MBP, MBzP, MEHP, MEHHP, and MEOHP in >70% of the samples, suggesting widespread exposure in this cohort to DEP, DBP, and DEHP. In multivariate models, higher levels of phthalate metabolites were associated with personal care products such as baby powder (MEP concentration increased by 17%); household products such as glass cleaner (MEHP concentration increased by 36%) and air fresheners (MEHHP concentration increased by 48%). Associations between the usages of plastic (MBP concentration increased by 22%) were also correlated with phthalate metabolites, indoor moisture was associated with several urinary metabolite concentrations.

Relevance: These preliminary results from the CHILD study suggest that infants in these four major Canadian cities are exposed to phthalates from multiple sources in the home, particularly personal care products and furnishings. As previously noted in Swedish populations, the higher concentration of BBzP (parent compound of MBzP) was associated with self-reported water leakage in the home. The question of whether or not such exposures increase the risk of developing asthma in childhood awaits declaration of the phenotype later in the life of this cohort.

6A: The Association of *Filaggrin* Mutations with Peanut Allergy is Unaffected by Atopic Asthma History

Programme A: Gene-Environment Interactions

Authors: **Yuka Asai**[1], Ann Clarke[1], Celia Greenwood[1], Reza Alizadehfar[1], Moshe Ben-Shoshan[1], Sara Brown[2], Linda Campbell[2], Deborah L Michel[3], Johanne Bussières[4], François Rousseau[4], Mary Fujiwara[1], Kenneth Morgan[1], Peter Hull[3], Alan Irvine[5], WH Irwin McLean[2]

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Supervisor: Dr. Ann Clarke and Dr. Celia Greenwood

Objective/Purpose: Peanut allergy is a source of significant morbidity and mortality, and its prevalence may be increasing. Recently, we described an association between peanut hypersensitivity and loss-of-function mutations in the gene encoding a skin barrier protein, filaggrin (*FLG*), which has been widely associated with atopic conditions. Due to the known association of peanut allergy with eczema and asthma, both of which have been linked to *FLG* mutations, it is difficult to determine if the relationship between *FLG* null mutations and peanut allergy is independent of underlying atopic disease; we set out to investigate this problem.

Methods: Using 674 subjects from a well-established Canadian peanut registry, and two control groups, 894 individuals sampled from the general population of Ontario, and 268 newborn blood samples from Quebec City, the odds ratios (OR) were calculated for the association between peanut allergy and *FLG* mutations. A variable for atopic asthma in the Ontario controls was created from available smoking and asthma data, and was compared with asthma reported in the peanut allergic cases. Logistic regression modeling was used to examine the effect of atopic asthma, age and gender on the relationship between peanut hypersensitivity and *FLG* mutations, as well as any interactions of these variables. This was followed by a sensitivity analysis examining the effect of asthma reporting error on the association between peanut allergy and *FLG* mutations, as well as the effect of peanut allergy status misclassification.

Findings: The relationship between peanut allergy and *FLG* mutations had a multivariate OR of 1.80 (95% CI: 1.24, 2.60), which remained stable throughout the analysis. This relationship did not lose significance when a 1% prevalence of peanut allergy in the control group was modeled with 100 iterations, with a significant OR with a 5^{th} and 95^{th} percentile of 1.71 and 1.90, respectively. However, the association became non-significant if a 20% peanut allergy case resolution was modeled.

Deliverables: We provide evidence that the association between *FLG* mutations and peanut allergy is independent of co-existence of peanut allergy with other atopic disease. This relationship is robust, despite error in the atopic asthma variable and peanut allergy status. The lack of *FLG* mutation presence in all peanut allergic individuals suggests future investigations in other barrier proteins or inflammatory mediators.

Relevance: The cutaneous barrier as the gateway to the atopic march has been widely discussed since the discovery of *FLG* defects in eczema. This theory raises the possibility that effective treatment of an impaired epidermal barrier may prevent allergic disease, including peanut allergy. This finding is of interest to all researchers and clinicians who treat allergic disease, and will be disseminated by traditional means and workshops with key stakeholders.

7A: Seventeen Novel Candidate Genes for Airway Hyperresponsiveness

Programme A: Gene-Environment Interactions

Authors: **Cynthia Kanagaratham**[1], Marino, R.[2], Camateros, P.[2], Radzioch, D.[1]
[1]Departments of Human Genetics, [2] Experimental Medicine, McGill University, Montreal
Supervisor: Dr. Danuta Radzioch

Objective/Purpose: Pulmonary airways naturally respond to bronchoconstricting stimuli by narrowing; however, in asthmatics this response is more forceful and can occur at lower concentrations of the stimuli. This feature, known as airway hyperresponsiveness (AHR), is an intermediate phenotype of asthma. The heritability of AHR has been shown through its familial aggregation and through the use of inbred strains of mice. However, since AHR is a polygenic trait, identifying the specific genes responsible for it proves to be a difficult task. Here we show that using a unique genetic panel ideal for the dissection of polygenic traits we are able to identify several candidate genes responsible for AHR.

Methods: We have at our disposal a panel of 35 AcB/BcA recombinant congenic strains of mice (F1), created by a cross between airway hyperreresponsive and hyporesponsive strains, A/J and C57BL/6J respectively. Each recombinant strain is fully inbred and contains 12.5% of the genome from one parental strain on the background of the other parental strain.

1. Informative strains, which exhibit the phenotype (Penh) of the minor genetic donor, were identified from the AcB/BcA panel and used to create F2 strains by backcrossing to the major genetic donor. F2 mice were phenotyped and genotyped for custom SNPs at 4Mbp intervals.
2. QTL and microarray analysis was done to identify candidate genes. The list of candidate genes was narrowed by selecting four genes containing non-synonymous mutations and expressed in the lungs, as well as genes differentially expressed in parental strains due to cis acting mutations.

Findings:

1. Three informative strains for AHR were identified from the AcB/BcA panel: AcB64, BcA85, and BcA86. The BcA86 cross was selected for F2 mapping and an F2 cross were created by backcrossing to parental strain, C57BL/6J.
2. Two QTLs containing a total of 232 genes were identified in the BcA86 F2 cross. Using our gene selection criteria, the 204 genes were narrowed to seventeen candidate genes. The authenticity of the function of these genes in AHR is currently in the process of being validated at the chromosomal and gene level.

Deliverables: Our approach provides a manageable list of candidate genes for AHR for which homologous genes can be identified in humans. This approach can be applied to identify candidate genes for other intermediate phenotypes of asthma, such as total and specific IgE and eosinophil counts.

Relevance: Genetic studies such as ours will help further AllerGen's mission to improve the quality of life of allergic/immune disease sufferers by allowing for targeted treatments and medications. Furthermore, identifying genes involved in allergy can be used to screen for individuals who are susceptible to developing disease. This allows these individuals at risk to take the necessary measures to prevent disease.

8A: Epigenetic Biomarkers of Established Allergic Disease in Peripheral Blood Mononuclear Cells

Programme A: Gene-Environment Interactions

Authors: **Michelle L. North**[1], Sarah M. A. Neumann[2], Lucia Lam[2], Lisa M. Steacy[3], Jenny Thiele[1], Michael S. Kobor[2,4], and Anne K. Ellis[1,3]
[1]Department of Biomedical & Molecular Sciences and Division of Allergy & Immunology, Department of Medicine, Queen's University, Kingston, ON. [2]Child & Family Research Institute and Centre for Molecular Medicine & Therapeutics, Vancouver, BC. [3]Allergy Research Unit, Kingston General Hospital, Kingston, ON. [4]Department of Medical Genetics, University of British Columbia, Vancouver, BC
Supervisor: Dr. Anne K. Ellis

Objective/Purpose: A significant proportion of the North American population suffers from seasonal allergic rhinitis. However, genomic DNA sequence can not explain why certain individuals develop allergic disease and others do not. Epigenetic modifications, such as DNA methylation, have begun to play a key role in explaining gene-environment interactions. We hypothesized that a genome-wide comparison of DNA methylation in atopics *vs.* controls would identify epigenetic modifications relevant to the allergic phenotype.

Methods: Fifteen adults with an established history of seasonal allergic rhinitis were enrolled in the study and allergy to rye grass was confirmed by skin prick test. Eight controls were enrolled and their non-atopic status to a panel of 12 common allergens, including rye grass, was confirmed by skin prick test. Heparinized peripheral blood was collected from all participants and a differential cell count was performed prior to further processing. Peripheral blood mononuclear cells (PBMCs) were isolated on an Accuprep® gradient and 100,000 cells were prepared on cytospin slides for post-processing differential cell counts. DNA was isolated from the remaining PBMCs and interrogated for genome-wide differences in DNA methylation using the Infinium Methylation 450K BeadArray and associated protocols (Illumina). Data quality control and initial normalization was done using GenomeStudio (Illumina). Linear regression and non-parametric t-tests were used on quantile normalized and filtered data to identify differentially methylated genes. False Discovery Rate was estimated using Bonferroni correction and all statistical analyses were performed using modified scripts from Matlab software (v.R2011a, The MathWorks, Inc.).

Findings: After correcting for age and gender, 77 sites had $p<0.001$ (not FDR corrected). Among the genes exhibiting >5% difference between controls and atopics, hypermethylated genes included those involved in complement/immune signaling and ion channels. Hypomethylated genes included proteases, cell adhesion molecules, transporters and eosinophil peroxidase.

Deliverables: PBMCs from atopic donors exhibit differences in DNA methylation compared to non-atopics. Epigenetic modifications may mediate gene-environment interactions relevant to the allergic phenotype.

Relevance: The identification of epigenetic biomarkers may lead to new diagnostic tests for allergies. Identifying differences in DNA methylation may lead to the development of better medications that reverse epigenetic modifications that promote and sustain allergic disease.

9A: Preliminary Results from a Planned Meta-Analysis of Genome-Wide Association Studies of Lung Function in Children

Programme A - Gene-Environment Interactions

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Supervisor: Dr. Lyle J. Palmer

Objective/Purpose: Lung function measures, such as forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC), are complex traits that are important factors associated with respiratory and other health outcomes (such as asthma). Few studies have reported genome-wide association (GWA) study results for lung function in children. We have recently formed an international consortium to investigate the genetics of lung function in childhood. A primary aim of the consortium is to investigate the genetic determinants of longitudinal changes in lung function throughout childhood. We describe in this poster the consortium and its goals, and present preliminary data from the largest cohort in the consortium - the Avon Longitudinal Study of Parents and Children (ALSPAC).

Methods: ALSPAC recruited pregnant women and has collected detailed information on these women and their children over the preceding 22 years. Lung function measurements were collected at age 8 and 15 years. We performed gender-specific analysis of change in FEV_1 on 5,714 children from ALSPAC. The effect of change in weight and change in height in terms of changes in FEV_1 was assessed, adjusting for baseline weight, baseline height, active and passive smoking, and doctor diagnosed asthma. For the meta-analysis, linear mixed effects models with a random intercept and a random slope, will be used to test for associations between ~2.5 million imputed single nucleotide polymorphisms (SNPs) and longitudinal changes in FEV1 in 5 birth cohort studies: Copenhagen Studies on Asthma in Childhood (COPSAC), BAMSE, Generation R, INfancia y Medio Ambiente (INMA) study and ALSPAC. This analysis will include ~8,200 children by the end of 2012.

Findings: Initial epidemiological analysis in ALSPAC indicated that, for boys, increases over time in height ($p < 1x10^{-6}$), but not increases over time in weight (p=0.83), were associated with increase in FEV_1. For girls, both increase over time in height (p=0.002) and in weight (p=0.019) were associated with increase in FEV_1. Besides baseline height and weight, none of the other potential covariates were significant predictors.

Deliverables: A meta-analysed GWAS of cross-sectional and longitudinal lung function over childhood. In addition, we will perform look-ups of known adult lung function loci in our consortium, as well as look-ups of novel loci we discover in adult cohorts.

Relevance: The identification of novel genetic determinants of pediatric lung function has the potential to improve our understanding of the biological basis of normal physiology, as well as the pathophysiology of asthma.

10A: Functional Analysis of a TSLP SNP Associated with Asthma

Programme A: Gene-Environment Interactions

Authors: **Loubna Akhabir**, Andrew Sandford
University of British Columbia
Supervisor: Dr. Andrew Sandford

Objective/Purpose: TSLP is a cytokine secreted by epithelial cells in response to different stimuli such as TLR ligands or viruses. TSLP has been shown to be sufficient to initiate experimental allergic airway inflammation and its gene expression has been shown to be increased in murine and human asthmatic lungs. The binding of TSLP to its receptor on mast cells, dendritic cells and activated T cells results in the promotion of a Th2-type inflammation. rs1837253 was identified as a putative causal SNP based on consistent association data both from candidate gene and genome-wide association studies; as well as the absence of significant linkage disequilibrium with other SNPs in the region. The aim of this work was to perform functional assays to uncover the mechanism underlying the involvement of rs1837253 in asthma pathogenesis.

Methods: DNA samples from the lungs of asthmatics and controls were genotyped using TaqMan assays and quantitative PCR (qPCR) assays were performed to compare levels of gene expression between genotypes; and that for both TSLP isoforms. *In silico,* analysis was performed to predict binding of regulatory proteins to the SNP site. Electrophoretic mobility shift assays (EMSA) were performed to test for potential differential binding of a regulatory protein to the SNP site. Sample preparation for mass spectroscopy is in progress.

Findings: qPCR did not demonstrate differential gene expression between phenotypes (fatal asthma or non-asthma) or between genotypes. This was most likely due to a low N and the fact that these data were solely for the short isoform as we were unable to detect high enough levels of the long isoform. Efforts are underway to increase production of the TSLP long isoform by stimulation of airway epithelial cells, increase the number of samples as well as pursue samples from a different source (blood). Preliminary EMSA data showed binding of a nuclear protein derived from A549 cells to the T allele and little or no binding of the C allele. To date, no candidate from *In silico* analysis was able to compete away the observed binding.

Deliverables: The objective of this research is to explain the asthma association of a singleton SNP 2.5 Kb from the *TSLP* gene and participate in the identification of novel therapeutic targets. The identification of the mechanism of involvement of this SNP will be a novel finding of considerable importance, as this SNP is the only TSLP variant that came up significantly in multiple genome-wide association studies of asthma.

Relevance: The ultimate goal of these functional studies is to reach a greater understanding of the molecular pathogenesis of asthma and eventually pave the way for novel therapies targeting the source of inflammation, rather than life-long therapies aimed at dampening inflammation and easing symptoms. The findings from our study will be amenable to publication in medical journals, and thus communicated to clinical scientists and other researchers, to complement our research findings and ultimately target our candidate gene for therapeutics.

11A: Exploring the Functional Role of *ORMDL3* in Innate Immunity

Programme A: Gene-Environment Interactions

Authors: **Karolynn Hsu**, Stuart Turvey
Child & Family Research Institute
University of British Columbia
Supervisor: Dr. Stuart Turvey

Objective/Purpose: *ORMDL3* has been linked with the risk of childhood asthma in multiple genome-wide association studies and candidate-gene studies. *ORMDL3* may function as a negative regulator of endoplasmic reticulum (ER) calcium homeostasis and genetic polymorphisms in asthma patients, which may result in higher levels of *ORMDL3*. I hypothesize that increased *ORMDL3* expression results in a heightened inflammatory response. The objective of my project is to establish the relationship between *ORMDL3* expression levels and inflammatory response *in vitro.*

Methods: Confirm expression of *ORMDL3* in airway epithelial cell lines by quantitative PCR (qPCR).

1. Manipulate *ORMDL3* expression levels in these cell lines with an over-expression plasmid or siRNA. Expression levels are verified by qPCR and Western blot.
2. Determine if over-expression or knockdown of *ORMDL3* affects cellular inflammatory response in response to stimulants such as IL-1β, TNF-α, and flagellin. Pro-inflammatory cytokine production is determined by ELISA.

Findings: Expression of *ORMDL3* was successfully manipulated using *ORMDL3*-specific siRNA or pEGFP-ORMDL3 over-expression plasmid. No difference in innate inflammatory response – production of IL-6 or IL-8 – was observed between conditions. A PCR-array showed that some genes are differentially expressed when *ORMDL3* expression is knocked down.

Deliverables: Altering *ORMDL3* expression levels does not affect IL-6 or IL-8 cytokine production in airway epithelial cells. However, changes in *ORMDL3* expression may correlate with differential expression of other genes involved in asthma or allergy.

Relevance: The results of this investigation help to further our understanding of functional genomics and asthma pathogenesis. This research provides insight into the relationship between *ORMDL3* expression levels and inflammatory response. Although changes in *ORMDL3* expression do not affect pro-inflammatory cytokine production, it may influence other immune responses. If *ORMDL3* does have a significant role in airway inflammation, future drug therapy targets may include *ORMDL3,* or molecules involved in immune response. In such cases, *ORMDL3* will have implications in pharmacogenomics, which uses an individual's genotype to optimize drug therapy. Understanding the pathogenesis of asthma and allergic diseases will therefore be important in the development of new treatments that address the initial triggers, rather than final symptoms, of these diseases.

12A: Evaluation of Exhaled Nitric Oxide Measurements in Infants at Three Months and Twelve Months of Age

Programme A: Gene-Environment Interactions

Authors: **Nadia Rampersad** RRT, **Colleen Keast** RRT, S Stanojevic PhD, S Balkovec RRT, R Jensen RRT, S Kang RRT, F Ratjen MD PhD, P Subbarao MD MSc
The Hospital for Sick Children, Toronto, Canada
Supervisor: Dr. Padmaja Subbarao

Objective/Purpose: It has previously been shown in adults that high levels of nitric oxide in exhaled breath are associated with airway inflammation. Exhaled nitric oxide (eNO) can be measured non-invasively starting in infancy; however the relevance of eNO values in the infant population, with respect to airways disease, is not well-established. Our aim was to determine whether eNO values measured in a cohort of healthy infants demonstrated tracking during the first year of life.

Methods: Infant subjects were recruited as part the Canadian Healthy Infant Longitudinal Development Study (CHILD Study). A subset of infants attended infant pulmonary function testing at the Hospital for Sick Children. Children who completed infant pulmonary function testing at three months and twelve months were included in these analyses. Infant eNO was measured using a multiple-breath sampling technique during quiet tidal breathing, while the subject is either in a natural sleep state or sedated with Chloral hydrate (80 mg/kg). During the test the child inhales nitric oxide (NO)-free air, supplied by the Eco Medics DENOX88 unit, *via* a facemask covering their oral and nasal cavities. Using the Eco Medics CLD88sp eNO analyzer, a stream of their exhaled breath is aspirated during each one-minute trial and the concentration of eNO is calculated with the Eco Medics Spiroware breath-by-breath analysis program. The correlation between three month and twelve month measurements was calculated using the Spearmen Correlation Test.

Findings: In total, 69 subjects were successfully tested for eNO values at three months; 16 of these subjects also attended a 12-month visit. There was no relationship between three month eNO and 12 month results (Spearman Correlation 0.185, p=0.49). Furthermore, three month eNO results were not predictive of 12 month results. In other words, children with elevated eNO results in early infancy did not necessarily also have elevated eNO at 12 months.

Deliverables: Exhaled Nitric Oxide data from the CHILD study will improve our understanding of these measurements in early childhood, and will help determine whether or not this test is clinically useful in infancy. These data will also be used to generate reference values, which will improve how clinical data are interpreted.

Relevance: Based on the findings from this preliminary evaluation, eNO values at three months and 12 months of age are not associated. These findings are limited given the small number of subjects that had successful eNO testing at both three and 12 months. Continued follow-up is needed to understand the relevance of the eNO values during the first year of life.

13A: Diesel Exhaust and Neurogenic Airway Inflammation

Programme A: Gene-Environment Interactions

Authors: **Francesco Sava**, Mandy Pui, Christopher Carlsten
Air Pollution Exposure Laboratory, University of British Columbia
Supervisor: Dr. Christopher Carlsten

Objective/Purpose: Air pollution is associated with adverse respiratory outcomes in at-risk populations such as asthmatics. Diesel exhaust (DE) is a major contributor to air pollution's detrimental effects and has been associated with asthma exacerbations and rhinitis. Airway inflammation is a central component to the pathophysiology of asthma exacerbation and is, in part, caused by neuropeptides released at nerve endings. One of the neuropeptides classically associated with this "neurogenic inflammation" (NI) is calcitonin gene-related peptide (CGRP). It is possible that DE exacerbates asthma through release of this peptide; this relationship has been suggested *in vitro* and in animal studies, but never formally in human studies. In the present study, we aim to quantify NI associated with controlled exposure to diesel exhaust in human asthmatic upper airways. We hypothesize that the NI marker CGRP will be increased in nasal lavage (NL), after acute controlled DE exposure in asthmatic subjects.

Methods: To investigate our hypotheses, asthmatic subjects and healthy controls have been exposed to freshly generated DE ($PM_{2.5}$ concentration 300 µg/m³) and filtered air (FA), each for 2 hours within an order-randomised double-blind crossover design. NL samples are taken at baseline and at 6 and 30 hours post-exposure. We detected and quantified the concentration of CGRP using a high-affinity competitive ELISA kit.

Findings: Data for nine subjects (6 asthmatic and 3 healthy controls) is presented. NL fluid CGRP concentration trends higher, at both 6 and 30 hours post-exposure, in the DE condition compared to FA (mean (SD) CGRP, in pg/mL. in DE vs FA: at 6 hours = 1.57 (0.2) vs 1.17 (0.4), p=0.09; and at 30 hours = 1.70 (0.2) vs 1.29 (0.2), p=0.11). Adjusting for baseline level, CGRP concentration was higher, at 6 hours post-exposure, in the DE condition, compared to FA (CGRP = 0.45 (0.2) vs 0.01 (0.2) pg/mL, p=0.04); these CGRP increases were similar between asthmatics and healthy controls.

Deliverables: This data provides insight into the mechanism of DE-related effects on upper airways pathophysiology and suggests that CGRP and neurogenic inflammation may play an important role in both asthma and rhinitis observed in epidemiologic studies of air pollution. These results open the way to yet another unexplored pathway for DE-induced human airway inflammation.

Relevance: Ultimately these findings will allow better mechanistic understanding of the role of NI in airway pathophysiology associated with traffic-related air pollution exposure. Elevated markers of NI, like CGRP, could lead to biomarkers of susceptibility to air pollution in at risk populations. Moreover, NI could be a pharmacologic target in these populations. This is the first human controlled exposure study directly looking at DE and NI. This unique design allows for study of human physiology *in vivo* while better controlling for factors that may confound other study designs. These findings will be made public in national and international conferences as well as peer-reviewed journals and shared with regulatory agencies such as WorkSafeBC and Heath Canada.

14A: Integrated Study of 'Omics' Sciences to Characterize the Molecular Biology of Interleukin 1 Type 2 Receptor in Allergic Asthma

Programme A: Gene-Environment Interactions

Authors: **Vanessa T. Vaillancourt**[1], Valérie Gagné-Ouellet[1], Simon-Pierre Guay[2,3], Luigi Bouchard[2,3,] Catherine Laprise[1]
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Supervisor: Dr. Catherine Laprise

Objective/Purpose: Illustration of the relevance of the integration of the ''omics'' sciences by defining the role of the Interleukin 1 receptor type 2 (*IL1R2*) gene in allergic asthma.

Methods: *IL1R2* was investigated using expression study, genetics and epigenetics approaches. First, the expression study was performed with the U133A GeneChips (Affymetrix, Santa Clara, CA) (n=16 subjects). Genetic association was identified for single nucleotide polymorphisms (SNP) in *IL1R2* gene with atopy (n>5500 DNAs). The genetic association studies were performed with the FBAT or PLINK softwares according to the study type (family and case-control). Genes involved in *IL1R2* gene activities were selected by a literature review. Correction for multiple testing (Independent TagSNPs and phenotypes) was applied. DNA methylation was measured on bisulfate treated DNA and using the pyrosequencing technology at three different *IL1R2* regions (*IL1R2-CpG1i*, -CpG2i and -*CpG3i*) in the CpG Islands observed at the gene promoter locus (n=47 DNAs). These three regions (16 CpG dinucletodies) cover 90% of the CpG dinucleotides observed at this promoter locus.

Findings: We previously demonstrated that *IL1R2* gene was 2.5-times overexpressed in bronchial biopsies of allergic asthmatics as compared to controls. We also shown that *IL1R2* SNPs were associated with atopy in four Canadian/Australian independent studies (p=0.0007; n>5500). Moreover, we showed that SNPs in 12 out of the 13 genes involved in the *IL1R2* activities were associated with asthma related phenotypes in five populations from Canada, France and Australia (14/990 SNPs). SNPs in seven genes (*BACE1*, *SPI1, IL1R2, IL1A, ERAP1, IL1RAP, MMP2)* remained associated after statistical correction in at least one population. Finally, DNA methylation profile of the *IL1R2* promoter region showed that one CpG dinucleotide in *IL1R2*-CpG1i was hypomethylated in asthmatics (p=0.045) and in atopics (p=0.046) compared to controls. Hypomethylation profiles were also observed in children that were exposed *in utero* to tobacco (p=0.004 and p=0.007 respectively for *IL1R2-CpG1i* and *IL1R2-CpG2i* regions). This hypomethylation in asthmatics and children exposed to tobacco *in utero,* may lead to an increase of *IL1R2* mRNA expression and consequently of the protein level. This increase in IL1R2 receptor may contribute to a higher proportion of IL1 bound with IL1R2 which promotes Th2 pathway. Further studies are needed to confirm this hypothesis.

Deliverables: The results of the genetic associations, combined with those of the DNA methylation analyses, suggest that the genes involved in *IL1R2* activities play an important role in asthma and asthma-related phenotypes. Moreover, those results allow us to have an overall view of the role of molecular biology of this receptor in allergic asthma endotypes.

Relevance: An integrated “omics” study may help better define the impact and role of biomarkers in the disorder and increase our ability to find relevant therapeutic targets. To date, more than 300 genes are associated with asthma. The integration of different approaches allows us to translate this important information and increase our comprehension of the physiopathology of the disease. Moreover, it gets us closer to a more specific therapy.

15A: Postpartum Depression: An Independent Predictor of Wheeze in Preschool Girls

Programme A: Gene-Environment Interactions

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Supervisor: Dr. Anita L. Kozyrskyj

Objective/Purpose: Postpartum depression is a serious health concern affecting 13% of child-bearing women. A growing body of evidence links caregiver stress, in key developmental stages with poor child health outcomes. Previously, our AllerGen-funded research found an association between recurrent maternal distress from birth and childhood asthma. However, it is unknown if postpartum depression, as a time-limited condition, poses an increased risk for asthma or preschool wheeze. We sought an opportunity to determine if postpartum depression has an independent effect on the development of wheeze in preschool aged children.

Methods: Data were extracted from the Community Perinatal Care Trial. This included information regarding postpartum depression, child health outcomes, and possible confounding factors from 791 women and their children in Calgary. Boys and girls were analyzed separately. Logistic regression analysis was performed to investigate the association between postpartum depression and wheeze at age three. Models were adjusted for the confounding factors of distress in pregnancy, smoking, preterm birth, childcare outside of the home, exclusive breastfeeding, and vitamin use.

Findings: In crude analysis, smoking after pregnancy (Odds Ratio [OR]: 3.37, 95% CI: 1.32-8.57) was associated with wheeze in boys. Postpartum depression (OR: 4.68, 95% CI: 1.20-18.3) and severe distress in pregnancy (OR: 4.41, 95% CI: 1.15-16.9) were crude significant predictors of wheeze in girls. When adjusted for confounds, postpartum depression remained a significant predictor of wheeze in girls (OR:4.70, 95% CI:1.12-19.8). When adjusted for prenatal vitamin use, this association became non-significant. There were no significant predictors of wheeze in boys in adjusted analysis.

Deliverables: Maternal depression limited to the postpartum period may be a risk factor for preschool wheeze in girls. Our findings also suggest that prenatal vitamin use may mediate this pathway. Although this hypothesis requires further study, women experiencing postpartum depression are more likely to have nutritional deficiencies during pregnancy, which have also been linked to asthma development.

Relevance: This hypothesis-generating research suggests a postnatal stress-nutrition pathway to preschool wheeze or asthma. Health initiatives which target maternal mental health after birth have the potential to reduce the risk of future wheeze in the child.

16A: Childhood Allergic Rhinitis, Traffic-Related Air Pollution, and the Role of Genetic Variability in the Oxidative Stress Pathway: Results from the TAG Study

Programme A: Gene-Environment Interactions

Authors: **Elaine Fuertes**[1], E. MacIntyre[1], E. Melén[2], J. Heinrich[3], M. Kerkhof[4], G. Pershagen[2], U. Gehring[5], C. Carlsten[1]*, M. Brauer[1]*

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Supervisors: Dr. Christopher Carlsten and Dr. Michael Brauer; *contributed equally

Objective/Purpose: Whether or not traffic-related air pollution (TRAP) affects allergic rhinitis prevalence and exacerbation remains controversial, possibly due to the confounding effect of genetics and gene-environment interactions. In a pooled analysis of six birth cohorts, we examined the association between TRAP, allergic rhinitis and sensitization in children, as well as the influence of three single nucleotide polymorphisms (SNPs) related to detoxification and oxidative stress: rs1138272/Ala114Val and rs1695/Ile105Val in the *glutathione-S-transferase P1 (GSTP1)* gene and rs1800629/-308 in the *tumor necrosis factor (TNF)* gene.

Methods: For each SNP, a child was identified as a "mutant carrier" if they were not homozygous for the wildtype allele (dominant genetic model). Associations between traffic-related NO_2 (individually assessed using land-use regression or dispersion models) and each SNP on allergic rhinitis, as well as sensitization to common aeroallergens were assessed using logistic regression. Allergic rhinitis was defined based on a doctor diagnosis or report of symptoms (runny or blocked nose, itchy, red and watery eyes) at the 7 or 8 year follow-up. Elevated risk of disease was analyzed per 10μm/m^3 increase in NO_2, after adjustment for relevant covariates. Results are presented as [odds ratios (95% confidence intervals)]. Interaction terms were introduced into the full models to test for gene-environment interactions.

Findings: Pooled analyses indicate that exposure to NO_2 increases the risk of allergic rhinitis at age 7 or 8 years ([1.14 (1.00 – 1.29)] per 10μg/m^3 NO_2). No association was found for aeroallergen sensitization ([1.07 (0.95 – 1.22)] per 10μg/m^3 NO_2). Carriers of at least one mutant rs1800629 allele were at a significantly elevated risk of developing allergic rhinitis ([1.21(1.01, 1.46)]), regardless of TRAP exposure. No significant associations between TRAP and allergic rhinitis, or sensitization, were observed in any of the pooled analyses stratified by genotype. Accordingly, interaction terms between each SNP and TRAP, which tested for gene-environment interactions, were not significant in all cases (p-value ranged from 0.13 to 0.91).

Deliverables: The results of this large study, suggest that children exposed to TRAP and those with at least one mutant rs1800629 allele in the *TNF* gene, are at a higher risk of developing allergic rhinitis. However, the effect of TRAP on allergic rhinitis and sensitization does not appear to be modified by genetic variability in the *GSTP1* and *TNF* genes.

Relevance: By identifying vulnerable populations, this research can be used to drive the development of air pollution policies that protect the entire population, which is key to reducing the health, social and economic costs of allergic rhinitis. This study is especially unique as it contains greater statistical power than any allergic rhinitis gene-environment study to date. Results will be disseminated at conferences and in peer-review publications. Major observations will also be reported on the TAG project website and in policy-and practice-relevant research summaries. The international make-up of the project's collaborators also helps ensure that these findings are widely distributed and used to improve human health to their fullest extent.

17A: Epigenetic Study of *CX3CR1* in Asthma

Programme A: Gene-Environment Interactions

Authors: **Andréanne Morin**[1], Simon-Pierre Guay[2], Jacynthe Lacroix[1], Luigi Bouchard[2], Catherine Laprise[1]
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Supervisor: Dr. Catherine Laprise

Objectives/Purpose: Chemokine (C-X_3-C motif) receptor 1 (CX3CR1) is a seven-transmembrane G protein coupled receptor (GPCR) involved in cell adhesion and migration of inflammatory cells. Our group previously demonstrated that *CX3CR1* is underexpressed (2-fold) in bronchial biopsies of asthmatics, compared to control subjects. We also observed that *CX3CR1* single nucleotide polymorphisms (SNPs) are associated with asthma in the asthmatic familial collection from the Saguenay–Lac-Saint-Jean (SLSJ): one SNP is located in the upstream region (g.-20873G>A) and two coding SNPs (p.V249I and p.T280M) are located in the second exon. Since DNA methylation of CpG motifs have been associated with gene silencing, we hypothesized that methylation at CpGs within the *CX3CR1* gene locus may explain differential expression observed in asthmatics compared to control subjects without asthma and atopy.

Methods: DNA and RNA were extracted from whole blood (n=48), from the SLSJ familial asthmatic collection selected, according on their phenotypes (asthmatics/non-asthmatic controls) and genotypes (p.V249I/p.T280M). Gene expression was assessed by qRT-PCR and DNA methylation by pyrosequencing of the bisulfite-treated DNA.

Findings: No difference in *CX3CR1* expression was observed between asthmatic and non-asthmatic phenotypes (p=0.3535). This may indicate that the previously observed differential expression of *CX3CR1* in bronchial biopsies may be tissue specific. As expected, a significant difference in DNA methylation levels at the two coding SNPs loci (p.V249I and p.T280M (which alter CpG sites)) was observed ($p<0.0001$). There was no correlation between *CX3CR1*gene expression and DNA methylation levels. However, when samples were stratified according to the phenotype, a positive correlation between *CX3CR1* gene expression and DNA methylation levels was observed in healthy subjects ($r= 0.5140$, $p<0.03$), while a negative correlation was observed in asthmatics ($r =-0.4204$, $p<0.03$).

Relevance: These findings suggest that DNA methylation at CpG sites, within the *CX3CR1* coding region may be associated with *CX3CR1* expression and could differ based on phenotype (asthmatics/controls). Methylation analysis of CpG sites near the promoter CX3CR1 g.-20873G>A polymorphism will be done soon. Further studies are needed to better understand whether DNA methylation plays a role in the differential expression of *CX3CR1* observed in asthma.

Funding source: AllerGen

18A Maternal Experience of Childhood Neglect is Associated with Allergy in Children at Two Years of Age

Programme A - Gene-Environment Interactions

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Supervisor: Dr. Claudio N. Soares

Objective/Purpose: To examine whether maternal childhood trauma and/or prenatal depression are associated with allergy development in children at two years of age in a prospective cohort of Canadian-born pregnant women; to further determine whether findings from a parallel cohort of pregnant immigrant women (supported by AllerGen) are unique to this population.

Methods: Data from a prospective birth cohort examining the effects of maternal adversity on infant health outcomes (The Maternal Adversity Vulnerability and Neurodevelopment (MAVAN) study) were analyzed. Prenatal depression was assessed in early and late pregnancy using the Montgomery Asberg Depression Rating Scale (MADRS). The experience of mothers' abuse and neglect during childhood was assessed using the Childhood Trauma Questionnaire (CTQ). The diagnosis of asthma and allergy in the offspring of the MAVAN cohort was assessed at two years postpartum by maternal report.

Findings: Elevated MADRS scores at 24-36 weeks, but not at 12-23 weeks, gestation were significantly associated with allergy in children at two years of age ($\chi^2 = 9.86$, df = 1, $p = 0.01$). Prenatal depression, at any time, was not associated with asthma in children at two years of age. Maternal emotional neglect was significantly associated with allergy in offspring at two years of age ($\chi^2 = 5.75$, df = 1, $p = 0.01$). The rate of allergies in children born to mothers reporting emotional neglect during childhood was double what was reported by the non-neglected sub-group (21/166 *vs.* 9/172). Maternal physical neglect during childhood was also significantly associated with allergy in children at two years of age ($\chi^2 = 4.16$, df = 1, $p = 0.04$). None of the other CTQ sub-scales were associated with allergy or asthma in children at two years of age.

Deliverables: Mothers, who experienced emotional and physical neglect during childhood as well as depressive symptoms late in pregnancy, are more likely to report allergy development among their children. Ongoing analyses will determine whether altered cortisol reactivity underlies these associations and characterizes a longstanding, trans-generational dysregulation. Further comparisons within our immigrant cohort should highlight whether immigration poses an additional or unique risk for childhood allergy development, within the context of existing prenatal stress/depression and/or childhood trauma/neglect.

Relevance: A better understanding of the mechanisms underlying allergy development in children can lead to development of interventions – preventative or in early life, when plasticity in physiologic development is particularly favourable.

19A: Modulation of the Neonatal Immune System by Listeria Monocytogenes Vaccine Vector: Does it Exacerbate Hypersensitivity Pneumonitis Development Later in Life?

Programme A: Gene-Environment Interactions

Authors: **Sheka Yagub Aloyouni**, Ashley Sherrid, Charis-P. Segeritz, Bing Cai, Matthew Gold, Kelly McNagny, Tobias R. Kollmann
Child and Family Research Institute and University of British Columbia
Supervisor: Dr. Tobias R. Kollmann

Objective/Purpose: Our attenuated *Listeria monocytogenes* (*Lm*) vaccine platform induces a strong T_H1 response in neonatal mice, protecting against certain T_H2-type diseases such as asthma. We sought to determine whether the T_H1-polarizing effect of this vaccine vector inadvertently may exacerbate development of certain T_H1-driven allergic diseases like hypersensitivity pneumonitis (HP), upon future allergen challenge.

Methods: Neonatal mice were immunized intraperitoneally, with either the live or heat-killed *Lm* attenuated strain, or with saline. Six weeks after immunization, anesthetized mice were challenged intranasally with *Saccharopolyspora rectivirgula* antigen (SR-Ag) on three consecutive days per week for three weeks. HP disease correlates were subsequently analyzed, including: 1) counts of the bronchoalveolar lavage fluids (BALF), to determine the total number and types of infiltrating cells; 2) quantification of IL-12, IL-17A, and IFN-γ cytokine production using Luminex following *in vitro* restimulation of spleen and lung cells, with SR-Ag or heat-killed *Lm*; 3) measurement of antigen-specific IgG1 and IgG2a concentrations in serum via ELISA analysis.

Findings: Neonatal mice immunized with the live-attenuated *Lm* vaccine appeared to develop exacerbated HP. This was evidenced by increases in total cell count and lymphocyte percentage in BALF in *Lm* compared to the control group. Elevated IgG2a and decreased IgG1 in serum from the *Lm* vaccinated group also supports this finding. Immunization with heat-killed *Lm* did not appear to exacerbate HP.

Deliverables: Neonatal vaccination with *Lm* vectors may exacerbate later development of some T_H1-type diseases such as HP.

- *Lm* vaccine-dependent exacerbation of HP requires live, replicating *Lm.*

Relevance: Neonatal immunization with *Lm*-based vaccines provides protection from certain T_H2 biased diseases such as asthma. This vaccine platform does not cause T_H1 disease by itself; however, it may exacerbate development of some T_H1-biased diseases later in life. These preliminary findings are currently being tested in appropriate details in order to determine if early life immunization has immune modulatory effects that need to be taken into consideration.

20A: Exclusive Breastfeeding Protects Against *Clostridium difficile* Colonization by Promoting Lower Relative Abundance of Lachnospiraceae in Gut Microbiota: Implications for Atopic Disease?

AllerGen Programme A: Gene-Environment Interactions

Authors: **Meghan B. Azad**[1], Konya T[2], Koster B[2], Maughan H[3], Guttman D[3], Sears MR[4], Becker AB[5], Scott JA[2], Kozyrskyj AL[1] and the CHILD Study Investigators[4].
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Supervisor: Dr. Anita L. Kozyrskyj

Objective/Purpose: Breastfed infants are less likely to be colonized by *Clostridium difficile* (*C. difficile)*, an intestinal pathogen associated with childhood atopy and asthma. Breastfeeding also modifies the community structure of intestinal microbiota. The objective of this study was to investigate and characterize the potential role of gut microbiota in the association of infant diet and *C. difficile* colonization.

Methods: The study comprised a small sub-sample of 24 infants from the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort. At age three months, fecal samples were collected and mothers reported breastfeeding status (exclusive, partial or none). *Clostridium difficile* colonization was determined by PCR, and microbiota composition was characterized by high-throughput 16S rRNA sequencing. Associations were investigated using exact logistic regression.

Findings: Exclusively breastfed infants (n=10) were less often colonized by *C. difficile* than those who were partially or not breastfed (n=14) (20.0% vs. 71.4%, p=0.04). Exclusively breastfed infants also exhibited markedly different gut microbiota profiles, characterized by lower relative abundance of Lachnospiraceae (median 8.1% vs. 19.5%, p=0.04). Lower abundance of Lachnospiraceae predicted a reduced likelihood of *C. difficile* colonization ($p<0.05$); however, this association was eliminated (p=0.21) following adjustment for exclusive breastfeeding, suggesting a common pathway. Results were unchanged by correction for caesarean-section delivery and maternal atopy.

Deliverables: Our findings suggest that exclusive breastfeeding protects against *C. difficile* colonization, by promoting lower relative abundance of Lachnospiraceae in gut microbiota. Ongoing studies will aim to confirm these results in a larger sample of the CHILD cohort, with incorporation of additional sampling time points and exposure variables. Potential associations with preschool wheeze and atopy will also be investigated.

Relevance: *Clostridium difficile* is a clinically important pathogen. Our findings emphasize the impact of a modifiable exposure (infant diet) on *C. difficile* colonization, and suggest a novel mechanism for this association. Full understanding of this pathway could ultimately lead to new strategies for allergic disease prevention through dietary optimization of infant gut microbiota.

21A: Effect of Thymic Stromal Lymphopoietin (TSLP), Interleukin (IL)-33 and IL-25 on Cord and Peripheral Blood $CD34^+$ Hemopoietic Progenitor Cell (HPC) Differentiation

Programme A: Gene-Environment Interactions

Authors: Claudia CK Hui**,** **Sina Rusta-Sallehy,** Delia Heroux**,** Judah A. Denburg
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Supervisor: Dr. Judah A. Denburg

Objective/Purpose: The epithelial cell-derived cytokines, TSLP, IL-33 and IL-25, all play roles in eliciting T_H2 immune responses. TSLP, in the presence of IL-33, has been shown to directly activate mast cells and cord blood (CB) $CD34^+$ HPC, with release of T_H2 cytokines/chemokines. Recent research shows that TSLP, combined with IL-25 and IL-33, induces a population of multi-potent mucosal progenitors in mice, a phenomenon which supports our group's postulate of "*in situ* hemopoiesis" as a key element of allergic inflammation involving eosinophil/basophil (Eo/B) progenitors in human airways. Additionally, TSLP has been found to upregulate IL-25R surface protein expression on T_H2 cells, increasing IL-25-dependent T_H2 responses, demonstrating cross-regulation between TSLP and IL-25. The aim of the current study is to evaluate the effects of TSLP, IL-33 and IL-25 on the differentiation of human CB and peripheral blood (PB) $CD34^+$ HPC into eosinophils.

Methods: Purified $CD34^+$ HPC, isolated from fresh PB and CB, were cultured in semisolid methylcellulose culture in the presence of the hemopoietic cytokines, IL-3 (0.1, 1, 10ng/mL), IL-5 (0.1, 1, 10ng/mL) and GM-CSF (1, 10, 100ng/mL), to obtain the optimal concentration of each cytokine needed to give the highest number of Eo/B CFU. Additionally, purified $CD34^+$ HPC isolated from fresh PB and CB, were stimulated with rTSLP (1 and 10ng/ml), IL-33 (10ng/mL), IL-25 (10ng/mL) or Iscoves 2^+ for control and cultured in methylcellulose colony assays in the presence of suboptimal and optimal doses of hemopoietic cytokines, IL-3, IL-5, and GM-CSF. Following a two week incubation period at 37^oC and 5.0% CO_2, mean numbers of Eo/B CFU were enumerated (colonies were defined as ≥ 40 cells).

Findings: In our dose response pilot study, IL-3 (10ng/mL), IL-5 (10ng/mL), and GM-CSF (100ng/mL) resulted in the highest number of Eo/B CFU from CB CD34+ cells (n=3), whereas, for PB $CD34^+$ cells (n=3), the optimal dose, was found to be 1ng/mL for all three hemopoietic cytokines. Stimulation of CB $CD34^+$ HPC with TSLP (10ng/mL) significantly increased the number of Eo/B CFU in response to IL-5 (1ng/mL) compared to control (no TSLP) (7.9±3.56 vs. 3.6±2.48 n=5, $P<0.001$). Additionally, IL-33 (10ng/mL) and IL-25 (10ng/mL) promote Eo/B differentiation of CB $CD34^+$ cells, as seen by the increase in Eo/B CFU compared to control (9±1.70 vs. 5.5±1.87 Eo/B CFU; 16.5±2.49 vs 8±2.94 Eo/B CFU, n=3).

Deliverables: Stimulation of $CD34^+$ progenitor cells by TSLP, IL-33 and IL-25 promotes Eo/B differentiation. Our findings suggest key roles for TSLP, IL-33 and IL-25 in influencing PB and CB HPC eosinophilic lineage commitment in allergic inflammation and disease.

Relevance: This in vitro model adds to our understanding of the effects of keyT_H2-inducing cytokines (TSLP, IL-25 and IL-33) on $CD34^+$ Eo/B progenitor differentiation. Further investigations may lead to novel biomarkers or therapies for atopic disorders.

22A: Is Breastfeeding Protective Against the Development of Asthma or Wheezing in Children? A Systematic Review and Meta-Analysis

Programme A: Gene-Environment Interactions

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Supervisors: Dr. Teresa To and Dr. Sharon Dell

Objective: Although breastfeeding is strongly recommended for its many benefits, the association between breastfeeding and childhood asthma development remains controversial. Our objective was to systematically review and meta-analyze the association between physician-diagnosed asthma or wheezing development and complete or any breastfeeding.

Methods: Prospective cohort studies of preschool (4-6 years) and school-aged (7-9 years) children were identified from Medline (1948-June 2011) and Embase (1980-June 2011). Breastfeeding exposure for at least the first 3-4 months of life was defined as complete (breast milk as the only source of nutrition) or any (breast milk included in the diet). Outcomes were parent-reported physician-diagnosed asthma or wheezing. Risk of bias in included studies was assessed using the Newcastle-Ottawa scale. Data were analyzed using the Revman software package and adjusted odds ratios were meta-analyzed using random-effects models.

Findings: Ten studies enrolling 35,411 participants were included. Decreased odds of physician-diagnosed asthma or wheezing development at ages 7-9 years were identified for those who received complete breastfeeding [adjusted odds ratio (OR) 0.69, 95% confidence interval (CI): 0.58-0.83] and any breastfeeding (OR 0.53, 95% CI: 0.41-0.68) and at ages 4-6 years for those who received complete breastfeeding (OR 0.75, 95% CI: 0.61-0.93). Among the clinically-heterogeneous studies with outcome assessment at ages 4-6 years, any breastfeeding did not change the odds of physician-diagnosed asthma or wheezing (OR 1.08, 95% CI: 0.76-1.54).

Deliverables: Complete or any breastfeeding for at least the first 3-4 months of life was associated with lower odds of physician-diagnosed asthma or wheezing in children at ages 7-9 years and complete breastfeeding was associated with lower odds of physician-diagnosed asthma or wheezing in children at ages 4-6 years.

Relevance: These results strengthen support for the current recommendations regarding complete breastfeeding in infancy. The findings also suggest that evaluations of breastfeeding should be included in cohort studies of childhood asthma. Inclusion of greater numbers of cohort studies in future systematic reviews may allow additional subgroup analyses, including separate evaluations of children with atopic and non-atopic asthma.

II. PROGRAMME B: DIAGNOSTICS AND THERAPEUTICS

#	Trainee	Level of Study	Institution	Supervisor(s)	Abstract Title
1B	**Bahreinian,** Salma	Research Assistant	University of Alberta	Anita L. Kozyrskyj	Exploring the Modifiable Biological Risk Factors of Asthma in Adolescents
2B	**Nowak,** Dominik	UG	University of Toronto Mississauga	Paul K. Keith	Reproducibility of Neutrophil Percentages in Pooled and Non-Pooled Nasal Lavage Samples
3B	**Yifei**, Zhu	UG	Queens University	Anne K. Ellis	Analysis of Il-10 and TNF-A Levels in the Supernatant of Cultured Cord Blood Adherent Mononuclear Cells as an Indicator of Atopic Risk
4B	**Stanojevic,** Sanja	Research Assistant	The Hospital for Sick Children	Padmaja Subbarao	The Lung Clearance Index in Early Life is not Independent of Body Size: CHILD Study
5B	**Hirota**, Jeremy	PDF	University of British Columbia	Darryl A. Knight	The Airway Epithelium NLRP3 Inflammasome Mediates Innate and Adaptive Immune Responses
6B	**Pascoe**, Christopher	PhD	University of British Columbia	Peter D. Paré Chun Y. Seow	Force Adaptation during Simulated Breathing Manoeuvres in Vitro
7B	**Watson**, Brittany	MSc	McMaster University	Gail M. Gauvreau	Expression and Function of Nictonic Acetylcholine Receptors on Basophils
8B	**Xu,** Jie (Janet)	UG	University of British Columbia	Delbert R. Dorscheid	Expression and Modulation of Surfactant Protein D in the Airway Epithelium of Asthmatics
9B	**Bredo,** Graeme	MSc	University of Alberta	Lisa Cameron	Influence of Il-25 on Th2 Lymphocytes and Allergic Inflammation
10B	**Lo,** Bernard	MSc	University of British Columbia	Kelly M. McNagny	The Function of CD34 in Pulmonary Fibrosis
11B	**Singh**, Amritpal	MSc	University of British Columbia	Scott J. Tebbutt	Common Genomic Response Profiles in Peripheral Blood of Allergic Asthma and Rhinitis Subjects Undergoing Independent Allergen Exposure Challenges
12B	**Yamamoto,** Masatsugu	PDF	University of British Columbia	Scott J. Tebbutt	Changes in the Expression of MicroRNAs in Peripheral Blood following Allergen Inhalation Challenge
13B	**Asaduzzaman,** Muhammad	PDF	University of Alberta	Harissios Vliagoftis	Functional Inhibition of PAR_2 Alleviates Allergen-Induced Airway Hyperresponsiveness and Inflammation

#	Trainee	Level of Study	Institution	Supervisor(s)	Abstract Title
14B	**Carson**, Kaitlyn	UG	Dalhousie University	Jean S. Marshall	The Effect of Toll-like Receptor Activation During the Induction of Oral Tolerance in Mice
15B	**Gold**, Matthew	PhD	University of British Columbia	Kelly M. McNagny	Novel Functional Role of CD34 in Regulating Mast Cell Activation and Migration
16B	**Yang**, Jasemine	PhD	University of British Columbia	Delbert R. Dorscheid	Characterization of IL-13 Receptors in the Asthmatic Airway Epithelium
17B	**Mastrangelo,** Peter	Research Associate	University of Toronto	Richard Hegele	Nucleolin Inhibition: A Novel Anti-RSV Strategy
18B	**Sadatsafavi,** Mohsen	PhD	University of British Columbia	Carlo Marra	Use of Long-acting Beta Agonists With or Without Inhaled Corticosteroids and Adverse Asthma-related Outcomes: A Population-Based Nested Case Control Study
19B	**Singhera**, Gurpreet	Research Assistant	University of British Columbia	Delbert R. Dorscheid	Effect of Environmental Challenges on lL-33 Release by Airway Epithelial Cells
20B	**Malouf,** Bianca	MSc	University of British Columbia	Christopher Carlsten	Effect of Diesel Exhaust and Antioxidant Supplementation on Airway Oxidative Stress upon Controlled Exposure in Humans

1B: Exploring the Modifiable Biological Risk Factors of Asthma in Adolescents

Programme B - Diagnostics and Therapeutics

Authors: **Salma Bahreinian**[1], Ball GD[1], Vander Leek TK[1], Becker AB[2], Kozyrskyj AL[1]
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Supervisor: Dr. Anita L. Kozyrskyj

Objective/Purpose: Thirteen percent of Canadian adolescents are affected by asthma and its impact on quality of life. Stress is a proposed risk factor for asthma development. In a previous study, we found a positive association between high AL index (a composite biological measure indicating cumulative effects of long-term stress) and risk of new-onset asthma in boys. The purpose of this study was to determine a parsimonious subset of biological markers that predicted the development of asthma in adolescents.

Methods: This was a prospective evaluation of children recruited at 7-10 years in the nested case-control study of Asthma, Genes and Environment (SAGE) and followed until age 11-14. Asthma diagnosis was confirmed clinically by a pediatric allergist at both visits. The eight following biological indices were measured at age 9-11: systolic and diastolic blood pressure (SBP and DBP), waist-to-hip ratio (WHR), early morning fasting levels of insulin, total cholesterol (TC), high density lipoprotein cholesterol (HDL), dehydroepiandrosterone sulphate (DHEAS) and cortisol. Biomarkers were dichotomized into high *versus* low risk groups based on the distribution of the biomarkers within the population under study. The high risk group was the highest quartile (compared to other study participants) for the measures of SBP, DBP, WHR, Insulin, TC and cortisol and the lowest quartile for HDL and DHEAS. The cutoff points for high *versus* low risk groups were calculated for each sex independently.

Findings: Out of 327 participants followed until 11-14 years (80% follow up rate), 108 (33%) had prevalent asthma. Using logistic regression modeling, a combination of high TC and DBP predicted atopic asthma (adjusted OR 5.12, 95% CI 1.23 to 21.22) in adolescent boys, whereas high TC and cortisol predicted non-atopic asthma (adjusted OR 3.25, 95% CI 1.07 to 10.03). The later combination also increased risk of developing new-onset asthma at 11-14 years in boys (adjusted OR 4.58, 95% CI 1.42 to 14.73). In girls, high fasting insulin levels significantly increased risk of having prevalent non-atopic asthma (adjusted OR 13.72, 95% CI 1.27 to 148.48).

Deliverables: We demonstrated that sub-clinical levels of biomarkers in childhood increase risk of having asthma in adolescence. Moreover, the biomarkers that predict asthma are different among adolescent boys *versus* girls, as well as atopic *versus* non-atopic asthma.

Relevance: These findings highlight simple measures of modifiable risk factors that can be used by clinicians to predict asthma in adolescents. Future studies are recommended to test the potential protective effect of modifying these biological risk factors on asthma in the adolescents, with and without well-known and established asthma risk factors.

2B: Reproducibility of Neutrophil Percentages in Pooled and Non-Pooled Nasal Lavage Samples

Programme B - Diagnostics and Therapeutics

Authors: **Dominik A. Nowak**[1], Penelope Ferrie[2], Paul K Keith[2]
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[2]McMaster University, Hamilton, Ontario, Canada
Supervisor: Dr. Paul K. Keith

Objective/Purpose: Neutrophils are important in a host's immediate immune response. COPD, ozone, infection and other factors have been shown to increase neutrophils in blood, sputum and nasal lavage. Nasal lavage is used to collect cells and inflammatory mediators from the nasal cavity, and can be used quantify neutrophilic response to inhaled stimuli. The objective of this study was to compare the reproducibility of neutrophil percentage in a single sample lavage, SSL, versus multiple sample lavage, MSL (3 lavages 15 minutes apart and pooled).

Methods: This study was a randomized crossover trial involving nasal lavage performed on four visits, 7-10 days apart. Lavage method was alternated between SSL and MSL during these four visits. Participants included seven with perennial allergic rhinitis, seven with bilateral nasal polyposis and seven controls.

Findings: The mean (±SEM) neutrophil percentage was not significantly different between the two methods (87 ±7 for SSL, 90 ±4 for MSL, p=0.2). With a minimum total cell count (TCC) of ≥20, the neutrophil percentage intraclass correlation (ICC) was similar but not sufficiently reproducible for SSL at 0.588 compared to MSL at 0.641. For samples with TCC≥100, the neutrophil percentage ICC of SSL was 0.93 (excellent) and that of MSL was 0.676 (satisfactory). The evaluable samples for TCC≥100 were 60/84 for SSL and 67/84 for MSL. We previously demonstrated in the same experiment that a TCC cutoff of ≥100 cells gave excellent eosinophil percentage ICC for both methods (>0.8).

Deliverables: SSL ICC was superior to MSL in measuring nasal lavage neutrophil percentage.

Relevance: Quantification of upper airway inflammation *via* neutrophil and eosinophil counts has direct clinical and research benefits. SSL can be used to assess the effects of inhalant stimuli on the nasal cavity and aid in diagnosis of rhinitic conditions. Furthermore, it is a valuable tool to accurately quantify changes in inflammation. Researchers will be able to assess effectiveness of treatments based on the values of inflammatory markers given by nasal lavage.

3B: Analysis of IL-10 and TNF-α levels in the Supernatant of Cultured Cord Blood Adherent Mononuclear Cells as an Indicator of Atopic Risk

Programme B - Diagnostics and Therapeutics

Authors: **Yifei Zhu**, Jenny Thiele, MSc, Anne K Ellis, MD, MSc, FRCP(C)
Queen's University
Supervisor: Dr. Anne K. Ellis

Objective/Purpose: The anti-inflammatory cytokine Interleukin-10 (IL-10) plays a key role in regulation of immune responses. Multiple studies indicate that atopic risk is associated with decreased levels of IL-10 (Van der Velden et al., 2001; Koning et al., 1997). In this project, we investigated differences in IL-10 and Tumour Necrosis Factor-α (TNF-α) levels secreted by adherent mononuclear cells (MNCs) between cord blood samples from atopic *vs*. non-atopic mothers. We ultimately aim to establish a biomarker panel that identifies future atopic risk at birth.

Methods: Informed consent was obtained from mothers undergoing elective scheduled C-sections for the procurement of cord blood samples. Atopic or non-atopic status was self-reported. From these samples, MNCs were isolated via AccuPrep® gradient centrifugation and temporarily stored in liquid nitrogen. Upon thawing, adherent cells were separated and cultured in RPMI complete media with or without Interferon-γ (IFN-γ) at 1 ng/ml and Control Standard Endotoxin (CSE) at 10 ng/ml. The cells were incubated under these conditions at 37 °C and 5% CO_2 for 5.5-21 hours. Afterwards, supernatants were collected and aliquots were frozen at -80 °C. Sandwich-ELISA (eBioscience) was performed to detect the concentrations of IL-10 and TNF-α in the supernatants.

Preliminary Findings: In samples of non- allergic mothers examined thus far, IL-10 and TNF-α secretion was upregulated after 5.5-21 hours, when stimulated with CSE in comparison to controls. However, in the sample from an atopic mother, no IL-10 was detected after CSE stimulation. TNF-α levels increased two-fold in this sample after CSE stimulation when compared to the increase seen in the sample from the non-atopic mother. Statistical analysis is pending the results of upcoming experiments.

Deliverables: The cytokine level variations between samples from atopic and non-atopic mothers are consistent with the functions of each cytokine as it relates to atopy. IL-10 is an anti-inflammatory cytokine. The lack of IL-10 in the sample from the atopic mother suggests a decreased ability to regulate inflammation, and this is consistent with the symptoms of an atopic patient. TNF-α is a pro-inflammatory cytokine. The fact that levels of TNF-α were greater in the sample from the atopic mother, as compared to the sample from the non-atopic mother, suggests greater inflammatory response upon stimulation. However, more samples will be investigated to draw a final conclusion.

Relevance: If IL-10 and TNF-α can be established as biomarkers of atopy in cord blood, they would provide a useful and simple tool for the early diagnosis of atopy. The early detection of atopy will be beneficial for preventative treatment, and is aligned with AllerGen's vision to reduce the burden of allergic diseases.

4B: The Lung Clearance Index in Early Life is not Independent of Body Size Canadian Healthy Infant Longitudinal Development Study

Programme B: Diagnostics and Therapeutics

Authors: Sanja Stanojevic, Meghan Brown, Susan Balkovec, Renee Jensen, Felix Ratjen, Padmaja Subbarao
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Supervisor: Dr. Padmaja Subbarao

Objective/Purpose: The lung clearance index (LCI) has been shown to be more sensitive in detecting abnormal lung function than spirometry in children. It is widely assumed that LCI is independent of body size; however recent evidence from healthy infants suggests this may not be true. The aim of this study is to determine the association between body size and LCI in healthy children.

Methods: Children participating in the Canadian Healthy Infant Longitudinal Study (CHILD) were invited to participate in infant pulmonary function tests. In addition, healthy control subjects from 3-6 years of age were recruited from the friends and family of patients and staff at the Hospital for Sick Children. Multiple-breath washout was measured by mass spectrometry (AMIS 2000; Innovision A/S, Odense, Denmark) using 4% SF6 and 4% He as tracer gases. The MBW is a non-invasive test and can be measured at all ages with minimal co-operation from the subject. The LCI is a measure of decreased gas mixing efficiency, or ventilation inhomogeneity. Fractional polynomial regression was used to investigate the relationship between body size and LCI, allowing for non-linear relationships.

Findings: MBW testing was attempted in 91 subjects between the ages of 2 months and 6.5 years. A total of 12 healthy control infants did not complete testing due to a failure of sedation. Using univariate analysis, the LCI was found to decrease non-linearly with increasing age and height. In a multivariable regression model, age and sex were not independent predictors of LCI. In healthy infants and young children, the LCI was dependent on body size; height explained the greatest variability in LCI.

Deliverables: Data from healthy infants participating in the CHILD study, combined with data from three other centres around the world, will be used to derive prediction equations for the LCI. These equations will be an invaluable tool for the interpretation of LCI in young children.

Relevance: Since LCI is not independent of body size, appropriate reference equations are required for accurate interpretation of results, particularly in infancy. Ignoring this dependency on body size will lead to over-diagnosis of early lung disease in children with wheezy disorders and asthma. The MBW technique is now commercially available and the reference equations developed using data from the CHILD cohort will provide a vital tool for the interpretation of LCI in children with known and suspected asthma.

5B: The Airway Epithelium NLRP3 Inflammasome Mediates Innate and Adaptive Immune Responses

Programme B - Diagnostics and Therapeutics

Authors: **Jeremy A. Hirota**, Matthew Gold, Paul Hiebert, Kelly McNagny, Darryl A. Knight
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Supervisor: Dr. Darryl A. Knight.

Objective/Purpose: Our overarching objective is to characterize the inflammatory responses mediated by the airway epithelium NLRP3 inflammasome to various environmental stimuli. We used PM, a clinically relevant exposure, as a stimulus for NLRP3 inflammasome mediated IL-1β production. We hypothesized that the airway epithelium expressed a NLRP3 inflammasome that mediates immune responses to PM, including IL-1β, CCL-20, GM-CSF, and TSLP production and changes in dendritic cell phenotype, respectively.

Methods: Gene expression, immunoblots and confocal microscopy were performed to confirm the presence of NLRP3 and caspase-1 protein in primary human airway epithelial cells and archived airway sections. Mechanistic *in vitro* studies were performed by exposing primary human airway epithelium cultures to PM in the presence of silencing RNA for NLRP3, and assessing IL-1β, GM-CSF, CCL-20, and TSLP protein production. *In vivo* exposure of NLPR3 -/- and wild-type control mice to PM was performed with outcome measurements of lung IL-1β, GM-CSF, CCL-20, TSLP protein production, inflammatory cell count differentials, and mediastinal lymph node dendritic cell phenotype.

Findings: Gene expression, immunoblot and confocal microscopy confirmed that NLRP3 and caspase-1 genes and proteins are expressed in airway epithelial cells *in vitro* and *in situ*. *In vitro* PM exposure to primary human airway epithelium resulted in NLRP3 inflammasome mediated production of IL-1β. PM-induced epithelium production of CCL-20 and GM-CSF was sensitive to NLRP3 inhibition, suggesting that these activators of dendritic cells may be produced downstream of NLRP3 activation. There were no PM-induced changes in TSLP observed. *In vivo* PM exposure corroborated our *in vitro* studies and demonstrated NLRP3 dependent elevations of MHC Class II+hi/CD11C+ hi cells in mediastinal lymph nodes.

Deliverables: NLRP3 and caspase-1 proteins are expressed in airway epithelium and form a functional inflammasome that is activated by PM exposure, resulting in innate and adaptive immune responses. The resulting production of IL-1β from the airway epithelium is associated with elevations in CCL-20 and GM-CSF, known modifiers of dendritic cell function.

Relevance: Our data supports targeting the airway epithelium NLRP3 inflammasome or downstream IL-1β signaling pathways to i) inhibit airway dendritic cell activation and ii) reduce exacerbations of existing airway diseases triggered by poor air quality. Our work may have positive influence on public health policies targeting air quality and development of therapeutic options to treat individuals during periods of poor air quality.

Funding: Canadian Institutes for Health Research/AllerGen/MSFHR

6B: Force Adaptation during Simulated Breathing Maneuvers in Vitro

Programme B - Diagnostics and Therapeutics

Authors: **Christopher Pascoe**[1,4], Yuekan Jiao[4], Chun Y. Seow[2,4], Peter D Paré[3,4], Ynuk Bossé[4]
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Supervisors: Dr. Peter Paré and Dr. Chun Y. Seow

Objective/Purpose: To determine whether oscillations that more closely mimic *in vivo* breathing maneuvers can attenuate the force adaptation phenomenon.

Methods: In the present study, we applied force oscillations simulating the tension oscillations experienced by the wall of a 4th generation airway during tidal breathing with or without deep inspirations (DI) to trachealis airway smooth muscle (ASM). Tone was induced by carbachol (average of 20nM) and the force-generating capacity of the ASM was assessed at five minute intervals before and after carbachol administration using electrical field stimulations (EFS).

Findings: The results show that force oscillations applied prior to the introduction of tone had a small effect on the force produced by EFS (declined to 96.8%, $p>0.05$, and 92.3%, $p<0.05$, with and without DI respectively). The tone induced by carbachol transiently decreased after a DI and declined significantly ($p<0.05$) due to tidal breathing oscillations (25%). More importantly, these force oscillations did not prevent force adaptation (gain of force of 11.2 ± 2.2 vs. 13.5 ± 2.7 and 11.2 ± 3.0% in static vs. dynamic conditions with or without DI respectively). This is likely due to a dramatic decrease in the strain experienced by the muscle following tone induction (94% and 60% reduction in strain caused by tidal breathing and deep inspiration respectively, $p<0.05$).

Deliverables: The lack of effect of breathing maneuver simulations on force adaptation, suggests that this gain in ASM force may occur *in vivo* and contribute to the development of airway hyperresponsiveness.

Relevance: ASM tone induced by inflammation in asthma may increase the force generating capacity of ASM by the phenomenon known as force adaptation. If we target this tone through the use of anticholinergic agents or inhibitors of inflammation, we may be able to stop force adaptation and in the process decrease AHR in asthmatic subjects.

7B: Expression and Function of Nicotinic Acetylcholine Receptors on Basophils

Programme B - Diagnostics and Therapeutics

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Supervisor: Dr. Gail M. Gauvreau

Objective/Purpose: The cholinergic anti-inflammatory pathway regulates the innate immune response to protect the body from prolonged inflammation, through the release of acetylcholine which binds to nicotinic acetylcholine receptors (nAChR). The nAChR α-7 subunit has been thought to play a role in modulation of cellular inflammation, and nAChR activation *via* nicotinic agonists could be a novel target for the treatment of inflammatory diseases like asthma. This study examined basophil expression of nAChR α-4, 7, 1/3/5 subunits and the effect of the nAChR α-7 subunit agonist, ASM-024, on basophil function.

Methods: 100 mL of blood was drawn from 14 allergic donors and layered using Accuprep density gradient. The buffy coat layer was collected and erythrocytes were lysed with ammonium chloride. Basophil-enriched cells were stained with antibodies to identify the basophil population (CD45+FCεR1+) and surface expression of nAChR α-4, 7, and 1/3/5 subunits. Cells were fixed in 1% PFA and acquired with a FACSCalibur flow cytometer. A separate aliquot of basophil-enriched cells was re-suspended in Ca^{2+} free HBSS and incubated with or without ASM-024 at concentrations of 10^{-3} M to 10^{-6} M for one hour at 37ºC. Cells were then stimulated with 1ug/mL anti-IgE for one hour at 37ºC. Cell supernatant was collected and stored at -70°C and analyzed for histamine using an ELISA (IBL-America). Controls included PBS stimulation or heating at 90 ºC for 10 min to quantify spontaneous release and total histamine release, respectively. Data are reported as mean (± SD), and analyzed by repeated measures ANOVA and Tukey post hoc tests.

Findings: The nAChR α-4, α-7 and α-1/3/5 subunits were expressed on 14.4±20.7%, 10.6±11.3%, and 12.2±8.5%, of basophils, respectively, compared to 1.9±0.2% expression of the isotype control. Basophils spontaneously released 1.5±0.7 ng/ml histamine which significantly increased following anti-IgE stimulation to 3.62±0.9 ng/ml ($p<0.05$). Pre-incubation of cells with ASM-024 at 10^{-6}M, 10^{-5}M, 10^{-4}M and 10^{-3}M significantly inhibited IgE-stimulated release of histamine to 1.0±0.5 ng/ml, 1.3±0.8 ng/ml, 1.3±0.6 ng/ml and 1.0±0.5 ng/ml histamine, respectively ($p<0.05$).

Relevance: We demonstrated that basophils express nAChR subunits α-4, α-7, and α-1/3/5. Furthermore, we established that IgE-induced histamine release from basophils is significantly inhibited by treatment with ASM-024, suggesting the nAChR α-7 subunit is indeed functional on these cells. These data support the notion that nACh receptors could be novel targets for the treatment of inflammatory diseases.

8B: Expression and Modulation of Surfactant Protein D in the Airway Epithelium of Asthmatics

Programme B - Diagnostics and Therapeutics

Authors: **Jie (Janet) Xu**, Gurpreet K. Singhera, Delbert R. Dorscheid
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Supervisor: Dr. Delbert R. Dorscheid

Objective/Purpose: Surfactant protein D (SP-D) is a pattern recognition molecule which plays important roles in the innate immune system. It binds a broad spectrum of pathogens including viruses, bacteria and fungi, enhances phagocytosis through interactions with neutrophils and mononuclear phagocytes and modulates inflammation. Respiratory syncytial virus (RSV) is a leading cause of lower respiratory tract infections and has been linked to the etiology of asthma; SP-D has also been reported to play a role in the clearance of RSV. The objective is to characterize SP-D expression in asthmatic and non-asthmatic airway epithelial cells (AEC).

Methods: Human airway sections from asthmatics and non-asthmatics and sections of pseudostratified air-liquid interface (ALI) cultures grown from primary AEC were used to quantify SP-D by immunohistochemistry. Normal human AEC cell line (1HAEo-) were either treated with interleukin-13 (IL-13) or infected with RSV followed by total protein extraction. Protein levels of SP-D in these samples were then determined using Western blotting.

Findings: SP-D molecules were expressed in intact human airway sections from both asthmatic and non-asthmatic donors. SP-D expression was found to be 33% higher ($p=0.004$) in asthmatic airways compared to non-asthmatic airways. In the non-asthmatic ALI cultures, RSV infection induced a slight reduction whereas IL-13 treatment significantly induced a 60% reduction ($p=0.0002$) in SP-D expression.

Deliverables: Our data demonstrated that surfactant proteins are expressed differently in the airways of asthmatics as compared to non-asthmatics. The increased SP-D detected in the asthmatic airways may reflect an increased susceptibility and response to viral infections potentially due to dysregulated protein expressions, dysfunctional protein products, or chronic injury of airway tissue. However, this requires further study.

Relevance: Characterization of surfactant protein expression in the asthmatic airway will contribute to our understanding of the increased susceptibility to viral infection observed in asthma.

9B: Influence of Il-25 on Th2 Lymphocytes and Allergic Inflammation

Programme B - Diagnostics and Therapeutics

Authors: **Graeme Bredo**, Alexis Adams, Jessica Storie and Lisa Cameron
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Supervisor: Dr. Lisa Cameron

Objective/Purpose: Allergic responses are inappropriate immune reactions to normally mundane substances and are mediated by inflammatory cells such as T Helper 2 (Th2) lymphocytes. Th2 cells often express the G protein coupled receptor, chemoattractant receptor-homologous molecule, or CRTh2. Activation of Th2 cells, via receptors such as CRTh2, stimulates Th2 cells to produce cytokines important for allergic responses such as IL-4, IL-5, IL-13 and IL-10. Our lab has observed that Th2 cells are highly positive for IL-25R while naive CD4 cells show very little IL-25R. Aside from mediating Th2 cytokine production, little is known about the effects of IL-25 on Th2 cells. A more thorough knowledge of this receptor may provide a better understanding of initiation and amplification of allergic responses.

Methods: CD4 T cells were isolated and cultured with anti-CD3/anti-CD28 and IL-2 in Th2 conditions (Il-4, anti-IL-12 and anti-IFNg). CRTh2+ cells were isolated and propagated with weekly cycles of activation (anti-CD3/anti-CD28 and IL-2, 3 days) and rest (IL-2, 4 days). IL-25 expression was identified with microarray and validated by qRT-PCR and surface staining. Chemotactic activity of IL-25 was assessed with a modified boyden chamber assay.

Results: Microarray, comparing total RNA levels of freshly isolated CD4 cells (3 days on anti-CD3/anti-CD28 and IL-2) compared to CRTh2 cells (day 45), showed a 300-fold increase in mRNA levels for the IL-25R in Th2 cells ($p<0.05$), which was substantiated by qRT-PCR. Flow cytometry showed high IL-25R protein expression after the anit-CD3/anti-CD28 stimulation, while showing low IL-25R after resting with IL-2. When CRTh2 cells were assayed for chemotaxis using a modified boyden chamber, IL-25 showed a moderate chemotactic profile.

Conclusion: The findings presented show the IL-25R in high abundance on CRTh2+ Th2 cells and that IL-25 may mediate chemotaxis of Th2 cells. The significance of CRTh2 cells in allergic diseases and the association of IL-25R on these cells, suggests IL-25 has a role in allergic inflammation. To expand on these findings, our future work will examine the role of IL-25 on Th2 differentiation.

10B: The Function of CD34 in Pulmonary Fibrosis

Programme B - Diagnostics and Therapeutics

Authors: **Bernard C. Lo**, Matthew J. Gold, Erin Debruin, Michael R. Hughes, Kelly M. McNagny
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Supervisor: Dr. Kelly M. McNagny

Objective/Purpose: Pulmonary injury as a result of allergic or autoimmune reactions, environmental irritants, or infection requires controlled repair to restore normal lung tissue architecture and function. When wound healing becomes dysregulated, fibrosis can arise accompanied by persistent inflammation, accumulation of extracellular matrix (ECM), and unresolved tissue remodeling. Interestingly, the CD34 cell surface sialomucin has been implicated in several processes associated with the pathogenesis and resolution of lung fibrosis, including early inflammatory cell recruitment, maintenance of vascular integrity, and angiogenesis. Known as a classic marker for hematopoietic progenitor cells, CD34 is expressed on mast cells, eosinophils, dendritic cells, and vascular endothelia. CD34-deficient mice are resistant to models of allergic asthma, hypersensitivity pneumonitis, and colitis due to defects in hematopoietic cell trafficking. Our objective is to characterize CD34 expression and function in the development of pulmonary fibrosis.

Methods: Lung fibrosis was induced in wild type (WT) C57Bl/6 and *Cd34*$^{-/-}$ mice with a single intratracheal administration of bleomycin sulfate. Early airway inflammation was determined by examining bronchoalveolar lavage (BAL) fluid to enumerate and identify immune cell infiltration. On day 21, fibrosis was assessed by changes in collagen deposition and airway mechanics using lung plethysmography (flexiVent).

Findings: Preliminary studies show that loss of *Cd34* exacerbates disease progression as the CD34-deficient mice display more pronounced weight loss and mortality compared to the WT controls. BAL cell counts indicate that *Cd34*$^{-/-}$ mice have elevated inflammatory cell infiltration in the alveolar space. However, loss of CD34 did not alter recruitment of specific leukocyte populations. By day 21, *Cd34*$^{-/-}$ mice have increased accumulation of collagen and display reduced lung capacity and compliance; characteristics suggesting a more severe fibrotic phenotype.

Deliverables and Relevance: Human idiopathic pulmonary fibrosis (IPF) is a chronic and ultimately fatal disease without effective therapeutic strategies. The bleomycin model has been well characterized but has limitations in that it lacks some key pathological features of the human disease. Nevertheless, our findings indicate that CD34 function may be critical in the resolution of fibrosis. In other inflammatory disease models, CD34 has a role in the recruitment of eosinophils and mast cells and thus perpetuates disease pathology. In the bleomycin fibrosis model, it has been reported that vascular leakage, which contributes to early inflammation, and an inability to regenerate damaged endothelia, are two factors that can accelerate fibrosis. Since CD34 is highly expressed by vascular endothelial cells and has been used as a marker for newly formed endothelia, increased susceptibility of the *Cd34*$^{-/-}$ mice in the bleomycin model fibrosis may be associated with CD34's function in these key processes.

11B: Common Genomic Response Profiles in Peripheral Blood of Allergic Asthma and Rhinitis Subjects Undergoing Independent Allergen Exposure Challenges

Programme B - Diagnostics and Therapeutics

Authors: **Amritpal Singh**[2], Kam SHY[1,2], Ruan J[1,2], Gauvreau M[3], O'Byrne PM[3], Ellis AK[4,5], FitzGerald JM[2,6,7], Boulet L-P[8], Tebbutt SJ[1,2,6]

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Supervisor: Dr. Scott J. Tebbutt

Objective/Purpose: Individuals with allergic asthma (AA) and individuals with allergic rhinitis (AR) exhibit either an isolated early phase response or a dual response. The purpose of this study is to determine common genomic response profiles, in peripheral blood of AA and AR individuals, undergoing independent allergen exposure challenges.

Methods: In the AA cohort, eight isolated early responders (ERs) and six dual responders (DRs) underwent a cat allergen inhalation challenge using the Clinical Investigator Collaborative protocol. Whole blood was collected (PAXgene RNA tubes) immediately prior to the challenge (pre) and two hours post-challenge. In the AR cohort, nine ERs and five DRs were simultaneously exposed to ragweed pollen in the Environmental Exposure Unit. Whole blood was collected (PAXgene RNA tubes) before and after three hours of pollen exposure. Complete blood cell counts and differentials were also obtained. Whole blood transcriptome profiling was performed using Affymetrix GeneChip® Human Gene 1.0 ST Arrays. Preprocessing and filtering was performed using the 'farms' (Factor Analysis for Robust Microarray Summarization) package and statistical analyses were carried out using the 'samr' (SAM: Significance Analysis of Microarrays) package in R (statistical computing program). A median false discovery rate (FDR) of 10% was used to assess differential gene expression between pre and post challenge. Ingenuity Pathway Analysis (IPA) was used to determine top biological functions.

Findings: 821 and 1346 probe sets survived filtering within the AA and AR datasets, respectively. 629 of these probe sets were in common. SAM identified 92 (48 up- and 44 down-regulated) differentially expressed probe sets (DEPs) in AA subjects. 42 (12 up- and 30 down-regulated) DEPs were identified for AR subjects. 22 genes were common between these two lists, and included several that have previously been associated with allergic disease mechanisms. IPA indicated that inflammatory disease was the top biological function for both AA and AR DEPs.

Deliverables/Relevance: Our data suggests that peripheral blood transcriptome analysis can reveal common underlying molecular mechanisms in allergic asthma and rhinitis. Further analysis may reveal common genes that discriminate isolated ERs from dual responders. Such knowledge is important because the late phase allergic response is associated with chronic inflammatory disease.

12B: Changes in the Expression of MicroRNAs in Peripheral Blood following Allergen Inhalation Challenge

Programme B - Diagnostics and Therapeutics

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Supervisor: Dr. Scott J. Tebbutt

Objective/Purpose: In atopic asthmatics, airway allergic inflammation induced by airbourne exposure to allergens results in airway obstruction, hyperresponsiveness and remodeling. MicroRNAs (miRNAs) are small non-coding RNAs that can bind to multiple target mRNAs to regulate protein production. The objective of this study is to measure the miRNA profiles in blood cells taken from mild atopic asthmatics undergoing allergen inhalation challenge (AIC).

Methods: Seven subjects were recruited for AIC. In all subjects, more than 20% drops in FEV1 were observed after AIC. Venous blood was collected (EDTA tubes) immediately prior to challenge (pre) and 2 hours post-challenge. Total RNA was extracted using a Qiagen miRNA mini kit. A total of 734 miRNAs derived from miRBase were profiled using the nCounter Expression Assay (NanoString Technologies, Seattle). After data processing, initial filtering and normalization, moderated robust regression in the statistical computing program R was used to assess differential expression of miRNAs. An unadjusted p value of < 0.05 was considered significant in this exploratory analysis.

Findings: A total of 178 miRNAs were detected across all samples, pre- and post- challenge. Ten differentially expressed miRNAs were identified following AIC. Six miRNAs were upregulated and four were downregulated after AIC.

Deliverables: The AIC model may improve understanding of regulatory mechanisms associated with asthma. Some miRNAs were identified to be candidates for further investigation. Additional recruitment of subjects and further analyses will identify more specific biological pathways that may be relevant to response type, such as the late asthmatic response.

Relevance: This research acts as an initial step in identifying miRNAs and pathways involved in the allergic response to AIC. The discovery of the biological roles will allow for a better understanding of the epigenetic regulation by miRNAs in exposure to allergen. It will also indicate potential therapeutic targets that can be utilized to minimize the late asthmatic response induced by allergic inflammatory pathogenesis.

13B: Functional Inhibition of PAR_2 Alleviates Allergen-induced Airway Hyperresponsiveness and Inflammation

Programme B - Diagnostics and Therapeutics

Authors: **Muhammad Asaduzzaman**[1], Courtney Davidson[1], Ahmed Nadeem[1], Narcy Arizmendi[1], John Gordon[2], Morley Hollenberg[3], Harissios Vliagoftis[1]
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Supervisor: Dr. Harissios Vliagoftis

Objective: Protease-Activated Receptor 2 (PAR_2) is a G protein-coupled receptor activated by trypsin-like serine proteinases. PAR_2 activation has been associated with inflammation. We have previously shown that PAR_2 activation in the airways leads to airway hyperresponsiveness (AHR) and inflammation, thus implicating PAR_2 in asthma pathogenesis. We now hypothesize that functional inhibition of PAR_2 during allergen challenge would inhibit allergen-induced AHR and airway inflammation in a murine model of asthma.

Methods: Male BALB/c mice were sensitized intraperitoneally with ovalbumin (OVA) absorbed to aluminum hydroxide followed by two intranasal (i.n.) challenges with OVA. To investigate the role of PAR_2 in the development of AHR and allergic airway inflammation we administered a blocking anti-PAR_2 monoclonal antibody (SAM-11) or an isotype matched control antibody i.n. either before both challenges with OVA (prevention protocol) or before the second challenge only (treatment protocol). A control group received saline only for both sensitization and challenge. AHR was assessed 24h after the last challenge and airway inflammation 48h later.

Findings: OVA sensitization and challenge induced AHR and airway inflammation were compared to control mice treated only with saline. In particular, OVA treatment increased airway resistance in response to methacholine and airway inflammation as shown by the presence of a greater number of total cells and eosinophils in bronchoalveolar lavage (BAL), than saline treated animals. Increased levels of cytokines and chemokines were also induced in the lung tissues. In addition, we observed OVA-specific T cell proliferation *in vitro*. Administration of the anti-PAR_2 antibody, but not the isotype matched control antibody, during the challenge phase significantly inhibited OVA-induced AHR and all measurements of airway inflammation in both the prevention and treatment protocols.

Deliverables: We will further identify the mechanism of PAR_2 involved in allergic inflammation and develop new approaches for treating allergic asthma.

Relevance: Our data shows that administration of an anti-PAR_2 antibody during allergen challenge improves allergen-induced AHR and airway inflammation in mice. Therefore, topical PAR_2 blockade in the airways may be used as a potential treatment of allergic asthma.

14B: The Effect of Toll-like Receptor Activation during the Induction of Oral Tolerance in Mice

Programme B - Diagnostics and Therapeutics

Authors: **Kaitlyn R. Carson**, Matthew Tunis and Jean S. Marshall
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Supervisor: Dr. Jean S. Marshall

Objective/Purpose: Toll-like receptors (TLRs) are innate immune receptors that recognize conserved molecular patterns. Many foodstuffs have been shown to activate TLRs. The objective of our research was to determine if TLR activation can interfere with the induction of oral tolerance to foods.

Methods: Oral tolerance was investigated in BALB/c mice by feeding the egg protein ovalbumin (OVA).

Two groups of mice were fed OVA *ad libitum* in drinking water for one week, while a control group was fed normal water. During the OVA feeding, one group of mice was gavage fed with 1 mg of OVA mixed with 5-10 µg of the TLR2 activators Pam_3CSK_4 or MALP-2, or the TLR4 activator LPS. The second OVA-fed group was gavage fed with OVA alone. Following the initial week of feeding, all three groups were immunized intraperitoneally (i.p.), with 10 µg OVA-alum to elicit an antibody response. Two weeks later, all mice received an i.p. boost dose of 1 µg OVA. Blood samples were obtained one week after the boost. Anti-OVA IgG1, IgG2a, IgE and IgA antibody levels were measured by enzyme-linked immunosorbent assay (ELISA).

Findings: Oral tolerance was impaired by oral exposure to Pam_3CSK_4 and LPS, but was not altered by MALP-2. These findings highlight the complexity of oral tolerance development and the need to closely examine the TLR activation profile of foods or their microbial contaminants.

Deliverables: Foodstuffs that activate TLRs may interfere with the induction of oral tolerance to food proteins.

Additionally, bacterial infection concurrent with the introduction of a new food may affect oral tolerance induction. This information is important for our understanding of how oral tolerance is regulated and suggests approaches whereby allergy to foods might be prevented. However, it is currently limited to an animal model and needs to be validated in humans.

Relevance: These data suggest that greater attention should be paid to the TLR activator content of foods when they are first introduced, or if they are being used to induce oral tolerance therapeutically. This information is of interest to the food industry and regulatory agencies and will be disseminated via the AllerGen network and other interactions with receptor groups.

15B: Novel Functional Role of CD34 in Regulating Mast Cell Activation and Migration

Programme B - Diagnostics and Therapeutics

Authors: **Matthew Gold**[1], Lionel Samayawardhena[2], Catherine J. Pallen[2], Kelly M. McNagny[1]
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Supervisor: Dr. Kelly M. McNagny

Objective/Purpose: CD34 is a cell surface sialomucin used extensively as a marker of hematopoietic stem cells (HSCs) and vascular endothelia however little is known about its biological functions. Our lab first showed that CD34 is expressed on mature mast cells, and has subsequently been found on dendritic cells, eosinophils and fibrocytes, and that CD34 facilitates the trafficking of these cells *in vivo*. Intriguingly, loss of CD34 expression renders mice resistant to asthma, hypersensitivity pneumonitis (HP), ulcerative colitis, salmonella infection and intestinal tumor development. Our aims are to characterize CD34's role in mast cell function *in vitro,* in order to further understand our *in vivo* findings, as well as explore the practicality and efficacy of CD34-targeted therapeutics.

Methods: Using bone marrow mast cells (BMMCs) derived from wild-type and *Cd34*$^{-/-}$ mice, we assessed the role of CD34 in mast cell signaling and effector functions in response to FcεRI cross-linking or stimulation with stem cell factor (SCF), a c-Kit ligand. We used degranulation, cytokine release and *in vitro* migration assays as measures of mast cell functions. To explore the function of various domains of the CD34 protein we have reconstituted *Cd34*$^{-/-}$ BMMCs with retroviral expression vectors encoding the full-length, as well as cytoplasmic-truncated isoforms of the CD34 proteins. We have also produced cDNA microarray data to explore the expression profiles in resting and FcεRI-stimulated wild-type and *Cd34*$^{-/-}$ BMMCs.

Findings: We have shown that BMMCs derived from *Cd34*$^{-/-}$ mice have enhanced cytokine production following FcεRI stimulation, but display no difference in degranulation or calcium mobilization. Detailed biochemical analysis revealed increased phosphorylation in several key signaling intermediaries downstream of the FcεRI cross-linking, including proteins involved in the NF-κB pathway, Syk and the β and γ subunits of the FcεRI receptor complex. Confocal and FACS analysis has demonstrated that CD34 is also maintained within the cell in intracellular stores, that can be rapidly translocated to the cell surface following stimulation. In addition, we found impaired migration of *Cd34*$^{-/-}$ BMMCs in response to a stem cell factor (SCF) stimulus.

Deliverables and Relevance: Mast cells are key effector cells in the pathology of allergic inflammation and asthma. Our *in vitro* analysis of CD34 function in mast cell activity revealed a novel functional role of CD34 in regulating local inflammatory responses. Through increased understanding of novel regulatory pathways in mast cell function, we hope to facilitate the development of novel mast cell targeting therapeutics.

This work was funded by the CIHR, the AllerGen Network Centre of Excellence and the Michael Smith Foundation for Health Research.

16B: Characterization of IL-13 Receptors in the Asthmatic Airway Epithelium

Programme B - Diagnostics and Therapeutics

Authors: **Jasemine S. Yang**, A.D.R. Saunders, S. Allaherverdian, S.J. Wadsworth, G.K. Singhera, D.R. Dorscheid
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Supervisor: Dr. Delbert R. Dorscheid

Objective/Purpose: The airway epithelium serves as a defense barrier and suffers frequent injury as a result, requiring repair coordinate with inflammation. Interleukin-13 (IL-13) is known to be a key cytokine in mediating inflammatory and remodeling processes in asthma. The actions of IL-13 are mediated by IL-13 receptor α1 (IL-13Rα1) and IL-13 receptor α2 (IL-13Rα2). Our laboratory has demonstrated that IL-13 is critical to normal airway epithelial repair via signaling the release of HB-EGF and activation of EGF-R. Appropriate control of inflammatory and repair processes is tightly regulated by the balance of IL-13Rα1 and IL-13Rα2 expression and function in response to injury. In this current investigation, we studied the expression of IL-13Rα1 and IL-13Rα2 in normal and asthmatic airways. 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: Expressions of IL-13Rα1 and IL-13Rα2 in sections from normal and asthmatic lung tissue and differentiated air-liquid interface (ALI) cultures of primary normal and asthmatic airway epithelial cells (AEC), were determined via immunohistochemistry and quantified using ImagePro Plus via colour segmentation. Primary airway epithelial cells from normal and asthmatic donors were cultured in monolayers and subjected to mechanical wounding and IL-13 stimulation over a time course of 24 hours. The cultures were then lysed for protein and RNA extraction and IL-13Rα1 and IL-13Rα2 levels were detected via Western blotting and qRT-PCR respectively.

Findings: Immunohistochemical detection demonstrated that asthmatic airways, specifically in the epithelium, expressed significantly ($p<0.05$) higher levels of IL-13Rα1 compared to normal donors. Asthmatic airways also do not express significant levels of IL-13Rα2 and exhibit epithelial abnormalities. Cultured monolayer AEC from asthmatic donors continue to secrete IL-13 in excess relative to normal AEC. In addition to dysregulated IL-13 release, these cells demonstrate abnormal IL-13Rα2 expression and function with markedly impaired repair.

Deliverables: Our data indicates that expression of IL-13 receptors is dysregulated in the asthmatic epithelium and this contributes to the dysfunctional repair phenotype observed in asthma.

Relevance: Strategies directed towards restoring normal IL-13 signaling and balanced expression of IL-13 receptors in the asthmatic airway epithelium, may lead to the development of novel therapies.

17B: Nucleolin Inhibition: A Novel Anti-RSV Strategy

Programme B - Diagnostics and Therapeutics

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Supervisor: Dr. Richard Hegele

Objective/Purpose: To determine if chemical inhibition of Nucleolin can decrease experimental respiratory syncytial virus (RSV) infection *in vitro*. The rationale for this approach builds on the identification by our group of Nucleolin as a cellular receptor for RSV infection (Tayyari *et al*., 2011, Nature Medicine).

Methods: Increasing amounts of a small molecule, Nucleolin inhibitor compound, were added in a fluorescence focus assay for quantification of RSV infection in epithelial cell cultures, either before viral challenge (prophylactically) or after viral challenge (therapeutically).

Findings: Cells that received the Nucleolin inhibitor compound showed significantly decreased RSV infection, in comparison to cells that received equimolar concentrations in a control molecule. Decreased RSV infection, without overt cellular toxicity, was observed when the Nucleolin inhibitor compound was used either prophylactically or therapeutically in our assay.

Deliverables: The Nucleolin inhibitor compound is already known to be safe for human administration and has been used in clinical trials for other indications. After completion of additional *in vitro* work, we will proceed to test the Nucleolin inhibitor compound in an *in vivo* model system.

Relevance: This work serves as proof in principle, that binding to cell surface Nucleolin *via* a small molecule can disrupt Nucleolin's role as a cellular receptor to RSV. Secondly, this work will form the basis for further work *in vivo,* which, if successful, could be extended to human clinical trials.

18B: Use of Long-acting Beta Agonists With or Without Inhaled Corticosteroids and Adverse Asthma-Related Outcomes: A Population-Based Nested Case Control Study

Programme B - Diagnostics and Therapeutics

Authors: **Mohsen Sadatsafavi**[1], Mark J. FitzGerald[2], Carlo A. Marra[1], Larry D. Lynd[1]
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Supervisor: Dr. Carlo Marra

Objectives/Purpose: Whether or not long-acting beta-agonists (LABAs) are safe when combined with inhaled corticosteroids (ICS) in the management of asthma is uncertain. Based on these concerns, the U.S. Food and Drug Administration (FDA) has mandated companies who market these drugs to complete a series of large randomized controlled safety trials. We used administrative health records of the population of the province of British Columbia to assess the risk of hospitalization or death in regular users of ICS+LABA compared with regular users of ICS alone or LABA alone.

Methods: We constructed a cohort of asthma patients between 12 to 45 years from 1997 to 2007. Within this cohort, we matched patients with asthma-related hospitalization or death to up to 20 control subjects based on age, date of entry into the cohort, and up to six measures of asthma severity. We categorized individuals as regular users, non-regular users, or no-users of ICS, LABA, or ICS+LABA in the 12 months prior to the index date and calculated the risk ratio (RR) between comparison groups.

Findings: 127,179 patients entered the cohort, with average follow-up time of 6.64 years per person. There were 2,797 cases which were matched to 25,014 controls. Compared to regular use of ICS, regular use of ICS+LABA was not significantly associated with an increased risk of adverse asthma events (RR=1.03 [95%CI 0.79 - 1.35]). On the other hand, regular use of LABA alone was associated with a significant increase in risk compared to both regular use of ICS (RR=2.22 [95%CI 1.43 - 3.45]) or to ICS+LABA (RR=2.15 [95%CI 1.32 - 3.48]). Regular users of LABA had to receive ICS for at least three quarters of a year to have a risk comparable to non-regular LABA users. There were no differences between regular users of ICS+LABA who took their medications in separate inhalers compared to those who received ICS and LABA regularly in single inhalers (RR=0.89 [95%CI 0.55 - 1.44]). Also, there was no difference between the two single inhaler ICS+LABA products approved in Canada (RR for Symbicort vs. Advair=1.05 [95%CI 0.58 - 1.90]).

Deliverables: Regular use of ICS+LABA does not seem to be associated with an increased risk of asthma-related hospitalization or death. The regularity of ICS use in patients who take LABA seems to be an important factor in the prevention of adverse asthma events.

Relevance: Our study is one of the first attempts to explore the safety of combination ICS+LABA therapy using administrative data. Results of the FDA-required RCTs will not be available for the next six years, and there are already concerns that such RCTs might not have enough power to detect a moderately increased risk. The benefit of LABAs in reducing asthma symptoms is undeniable, and our results provide valuable information for patients, physicians, and policy makers with regard to the safety of LABAs when concomitant ICS use can be assured.

19B: Effect of Environmental Challenges on IL-33 Release by Airway Epithelial Cells

Programme B - Diagnostics and Therapeutics

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Supervisor: Dr. Delbert R. Dorscheid

Objective/Purpose: Interleukin (IL)-33 is a novel member of the IL-1 family with dual function, as a cytokine acting through activation of the ST2 receptor and as an intracellular nuclear factor with potential transcriptional regulatory properties. IL-33 is known to be expressed on bronchial epithelium and airway smooth muscle cells. As an “alarmin molecule” IL-33 induces either pro- or anti-inflammatory cascades. In this study we investigated the localization of IL-33 in diseased human airways and the effects of specific environmental challenges on IL-33 production and release.

Methods: Using immunohistochemistry (IHC) techniques, IL-33 expression was characterized in airway sections of normal and diseased airways (asthma, chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF)) from the JHRC Biobank using a semi-quantitative scoring tool. In an *in vitro* model, primary human bronchial epithelial cells (HBEC) were incubated with RSV, anti-Fas (DR2) Ab, or TRAIL (DR4/5 ligand) followed by collection of total cell lysates and supernatants. IL-33 was detected in these samples by either ELISA or Western Blots (WB).

Findings: IHC data demonstrated that IL-33 is predominantly expressed in the nuclei of basal epithelial cells in airway sections of all groups studied. Asthmatic airways had a significant increase in IL-33 when compared to COPD and CF airways. *In vitro* AEC experiments demonstrated higher IL-33 release from asthmatic AEC at baseline and after exposure to environmental challenges with respect to normal AEC. Compartmentalization of IL-33 between total cellular and nuclear fractions was altered with similar stimulation.

Deliverables: In summary, environmental challenges can induce the release of IL-33 from both normal and asthmatic AEC. Relative to normal AEC, asthmatic cells release significantly more IL-33 in response to environmental challenges.

Relevance: Understanding the effects of environmental challenges on the regulation of IL-33 in AEC, will aid in better control of allergic inflammation in asthma.

20B: Effect of Diesel Exhaust and Antioxidant Supplementation on Airway Oxidative Stress upon Controlled Exposure in Humans

Programme B: Diagnostics and Therapeutics

Authors: **Bianca Malouf**, M.J. MacNutt, P. Provencher, W. Chu, C. Carlsten
University of British Columbia
Supervisor: Dr. Christopher Carlsten

Objective/Purpose: Oxidative stress is thought to underlie negative health effects associated with exposure to diesel exhaust (DE). Our objective was to test, using exhaled breath condensate, the hypothesis that anti-oxidant supplementation attenuates DE-related changes in airway oxidative stress in those individuals with hyperresponsive airways.

Methods: Six subjects with airway hyperresponsiveness to methacholine completed a double-blinded, randomized, and counter-balanced crossover study of 3 exposure conditions, each for 2 hours and separated by a ≥2-wk washout period: (1) DE (300ug $PM_{2.5}/m^3$) with anti-oxidant (N-acetylcysteine 600mg 3x/day on the 5 days preceding and the day of the exposure) ["DEN"], (2) DE with placebo ["DEP"], or (3) filtered air with placebo ["FAP"]. Exhaled breath condensate (EBC) was collected at baseline and at 2hr, 6hr and 30hrs post-exposure. Airway oxidative stress was characterized by EBC levels of 8-isoprostane, as measured by liquid chromatography tandem mass spectrophotometry. 8-isoprostane levels at 2-, 6- and 30-hours after exposure initiation were each corrected for baseline levels, thus generating "delta" values. The differences in delta values between exposure conditions were assessed by paired t-testing between FAP and DEP and between DEP and DEN.

Findings: Chromatography required extensive optimization, which resulted in a faster solvent gradient from water to acetonitrile, and thus, in sharper peaks and shorter run times. Fragmentation and collision energy in the reaction cell was further optimized, improving abundance of daughter ions, increasing counts in transition from parent to daughter ion. Sensitivity was maintained by changing the electron multiplier voltage (EMV) on the electron multiplier horn, to 600watt. With the optimized chromatographic technique, there was a trend towards increased 8-isoprostane in DEP deltas relative to FAP deltas (and a reciprocal correction with DEN), but this trend in the mean values is complicated by large standard deviations; paired t-tests for FAP *vs*. DEP and for DEP *vs.* DEN, using this preliminary data, were non-significant. Table 1 shows the delta values for all time-points and conditions. Data collection and analysis is ongoing and an additional 20 patients will be added to this analysis prior to February 2012.

Table 1: Delta 8-isoprostane Levels (mean (SD), in pg/ml) At Given Interval from Exposure Initiation

	FAP delta	DEP delta	DEN delta
2h	14.14 (36.87)	30.34 (38.65)	16.96 (108.21)
6h	19.31 (32.01)	0.102 (12.27)	-30.67 (68.15)
30h	29.89 (64.40)	10.09 (15.72)	-13.57 (26.41)

Deliverables: Preliminary data from an ongoing study using unique methodology to characterize 8-isoprostane in EBC suggests that DE, freshly-diluted and aged to reflect high-ambient or occupational concentrations, may increase airway oxidative stress at an early timepoint and that this effect may be mitigated by anti-oxidant supplementation (N-acetylcysteine). However, conclusions are limited by the modest sample size which will be augmented in coming months.

Relevance: The results of our study will be directly communicated to WorkSafeBC, who has funded this study and has a policy of translating research findings to policy when feasible.

III. PROGRAMME C: PUBLIC HEALTH, ETHICS, POLICY AND SOCIETY

#		Trainee	Institution	Supervisor(s)	Abstract Title
1C	**Arrandale,** Victoria	PhD	University of Toronto	Linn D. Holness	The 2nd AllerGen Funded Skin-lung Workshop: A Summary of Occupational and Work-related Allergy and Asthma
2C	**Barrick**, Kendra	MSc	Queens University	Diane Lougheed	Compensated Work-related Asthma in Ontario: A Data Linkage Analysis
3C	**De Olim Rugginenti,** Carlo	MSc	Université de Montréal	Catherine Lemière	Assessment of Health Care Utilization of Workers with Work-related Asthma in Quebec and Ontario
4C	**Des Cormiers,** Annick	MSc	Université Laval	Louis-Philippe Boulet	Decisional Conflict in Asthma Management
5C	**Blood,** Roxanne	MSc	University of Alberta	Miriam Steward Malcolm King	Aboriginal Children with Respiratory Problems and their Parents: Health Inequities
6C	**Sadatsafavi,** Mohsen	PhD	University of British Columbia	Carlo Marra	Use of Complementary and Alternative Therapies in Patients with Asthma: Preliminary Results from a Prospective Study
7C	**Soller,** Lianne	PhD	McGill University	Ann Clarke	The Prevalence of Food Allergy among Aboriginals in Canada
8C	**Killorn,** Katie	MSc	Queens University	Diane Lougheed Patti Groome	The Work-related Asthma Screening Questionnaire (Long Version) (WRASQ(L)L: Reliability and Effect on Provider Behaviour
9C	**Mykhaylova**, Natalia	PhD	University of Toronto	Greg Evans	A Novel Way of Preventing and Managing Allergic Disease and Asthma: Real-time Index-based Monitoring of Environmental Factors
10C	**Nelligan,** Kathleen	UG	The Hospital for Sick Children	Sharon Dell	Who's Willing to Trade in the Family Dog? Socioeconomic Status (SES) Predicts Differential Avoidance of Asthma Triggers
11C	**Sadatsafavi,** Mohsen	PhD	University of British Columbia	Carlo Marra	Asthma Control in a Random Sample of Canadian Asthma Patients
12C	**Chow**, Bonnie Y.L.	PhD	Waterloo University	Susan J. Elliott	Determinants of Purchasing Behaviours of Allergy Affected Canadian Consumers: A Mixed Methods Approach

#		Trainee	Institution	Supervisor(s)	Abstract Title
13C	**Luo,** Jing C.	Research Assistant	University of Manitoba	Allan B. Becker	The Impact of Recruitment Methods on CHILD Study Enrollment
14C	**Pawlowski**, Alicia	MSc	University of Alberta	Anita L. Kozyrskyj	Mothers' Potential for Self-blame in Reaction to a Genetic Association Study
15C	**Unruh,** Claire	UG	University of Manitoba	Allan B. Becker	Use of Social Media in the CHILD Study

1C: 2nd AllerGen Funded Skin-lung Workshop: A Summary Occupational and Work-related Allergy and Asthma

Programme C - Public Health, Ethics, Policy and Society

Authors: **Victoria H. Arrandale**[1], D. L. Holness[1, 2]
[1] University of Toronto, Toronto, Ontario, Canada
[2] St. Michael's Hospital, Toronto, Ontario, Canada
Supervisor: Dr. D. Linn Holness

Objectives/Purpose: The connection between work-related allergic skin and respiratory disease continues to be an area of research interest. Many allergens in the workplace cause both skin and respiratory outcomes. Most workers have both skin and inhalation exposure, and animal models have shown that the skin can be an important route of sensitization. However, we still do not fully understand the role of skin exposure in the development of occupational allergic asthma. The 2nd AllerGen Skin-Lung Workshop sought to reconvene participants from a previous workshop (2005) in order to summarize the progress to date and to re-evaluate the research priorities.

Methods: Participants from the 2005 Skin-Lung Workshop, as well as attendees from the Occupational and Environmental Exposures of Skin to Chemicals (OEESC) meeting, were invited to participate in the workshop. The workshop consisted of three structure presentations (animal models and mechanisms, exposure assessment and epidemiology of skin-lung interactions) followed by an interactive large group discussion.

Deliverables: The workshop resulted in an updated list of research needs in the area of work-related skin and respiratory allergic disease.

The areas targeted for future research were:

- Development of skin exposure standards and/or exposure limits
- Improving the estimates of the burden of occupational allergic disease
- Improving knowledge translation around all potential effects of skin exposure
- Investigating cytokine profiling for the differentiation of respiratory and skin sensitizers
- Investigating the role of skin barrier function in skin exposure and skin-lung interactions
- Improving the understanding of the relationship between skin and inhalation exposures
- Investigating the potential relevance of skin-lung interaction in environmental exposure scenarios

Relevance: The research priorities resulting from this multi-disciplinary and multi-national workshop were generated by a diverse group with considerable knowledge and expertise. We will begin to address these knowledge gaps, both individually and in collaborative groups. Improved understanding of the connection between the skin and the lungs in occupational disease will lead to reduced exposure, fewer cases of work-related allergic disease, and improved compensation for affected workers.

2C: Compensated Work-related Asthma in Ontario: A Data Linkage Analysis

Programme C - Public Health, Ethics, Policy and Society

Authors: **Kendra Barrick**[1,3],S. Dostaler[1,2] ,L. E. Lévesque[3,4,5],W. Pickett[3,8],T. To[7],G. Liss[6], S. M. Tarlo[6],

[1]Asthma Research Unit, Kingston General Hospital; [2]Department of Medicine, Queen's University, [3]Department of Community Health & Epidemiology, Queen's University; [4]Kingston, Frontenac, Lennox and Addington Public Health; [5]ICES-Queen's; [6]Department of Medicine, University of Toronto; [7]Child Health Evaluative Sciences, The Hospital for Sick Children; [8]Clinical Research Centre, Kingston General Hospital

Supervisor: Dr. Diane Lougheed[1,2,3,5,8]

Objective/Purpose: Asthma is the most common chronic respiratory disease in Canada and affects approximately 8% of adults. Ten to 25% of adult asthma cases are believed to be caused or aggravated by occupational exposures (Tarlo, 2008). Work-related asthma (WRA) is increasing in both incidence and prevalence and has been demonstrated to be under-recognized and under-reported in Ontario (Tarlo, 2008). WRA includes occupational asthma (OA) and work-aggravated asthma (WAA). The primary objective of this study is to estimate the prevalence of compensated WRA among individuals with asthma in Ontario from April 1998 to March 2002 as well as profile patterns of WRA by demographic, geographic and temporal factors. The secondary objective is to examine factors associated with a WRA claim being captured as asthma, in the Institute for Clinical Evaluative Sciences (ICES) Ontario Asthma Surveillance Information System (OASIS), for various groups of WRA claims.

Methods: To estimate the prevalence of compensated WRA among individuals with asthma in Ontario, data from the Ontario Workplace Safety and Insurance Board's (WSIB) Occupational Disease Information Surveillance System (ODISS) were linked to ICES OASIS for 1998 to 2002. ODISS is a cumulative database that provides information on disease claims submitted to the WSIB. OASIS is a population-based database that uses a validated algorithm of administrative data to identify individuals with asthma in Ontario aged 0 to 99 years since 1991. The data linkage between ODISS and OASIS involved two steps: 1) data from ODISS was linked to the Registered Persons Database using name, date of birth and sex to identify the encrypted Health Card Number (HCN); 2) the selected records were then linked to OASIS *via* the HCN. The prevalence of compensated WRA was profiled temporally by fiscal year, geographically by Local Health Integration Network (LHIN) and rurality, and demographically by age and sex. To determine factors associated with a WRA awarded claim being captured as asthma in OASIS, separate multiple logistic regressions were performed to examine the influence of numerous factors on the capture of WRA, OA and WAA awarded claims as asthma in ICES OASIS.

Findings: Analyses demonstrate an overall prevalence rate for compensated WRA, OA and WAA of 47.95, 41.56 and 6.39 cases per 100,000 individuals with asthma. The prevalence of WRA and OA increased from 8.9 to 16.1 cases per 100,000 and from 7.50 to 15.04 cases per 100,000, whereas the prevalence of WAA was fairly constant. The prevalence of WRA was the highest in females and in individuals aged 35 to 44. The prevalence of WRA was the highest in the Hamilton Niagara Haldimand Brant LHIN and in urban areas compared to rural areas.

Relevance: This project will provide insight into the burden of WRA in Ontario, by profiling the epidemiology of compensated WRA amongst individuals with asthma as well as informing researchers and policy makers of the feasibility of using WSIB-ICES data linkage as a surveillance system for compensated WRA to monitor provincial WRA trends.

3C: Assessment of Health Care Utilization of Workers with Work-related Asthma in Quebec and Ontario

Programme C - Public Health, Ethics, Policy and Society

Authors: **Carlo de Olim** BSc[1], C. Tremblay BSc[1], M. Ribeiro MD[2], A. Forget MSc[1], L. Blais PhD[1], T. To PhD[3], S.M. Tarlo MD[2]; C. Lemière MD, MSc[1]
[1] Hôpital du Sacré-Coeur, Université de Montréal, [2]University of Toronto,
[3]The Hospital for Sick Children, University of Toronto
Supervisor: Dr. Catherine Lemière

Objective/Purpose: Work-related asthma is associated with an important use of medical resources. The aim of this study is to compare the health care utilization (visits to the physician, emergency room visits and hospitalizations for asthma and all causes) before and after the diagnosis of work-related asthma (WRA) in subjects with occupational asthma (OA) and work-exacerbated asthma (WEA) from Quebec and Ontario.

Methods: We conducted a retrospective cohort study with subjects followed between 2000 and 2007 at two tertiary centers, one in Montreal (Quebec) and the other in Toronto (Ontario). Clinical data was extracted from the clinical charts. Data on health care utilization was collected by linking the workers' health care insurance number to the administrative database of government agencies. The rate of health care utilization was calculated for each sample and stratified by etiological agents (high (HMW) and low molecular weight (LMW) agents). We determined the statistical differences for the rates of Quebec and Ontario by using Student's T Test.

Findings: A total of 436 subjects from Montreal and 128 from Toronto were included in this study. The reductions in the rates of ER visits for asthma in subjects with OA during the year following the diagnosis were higher in Quebec (all cases: 17.2% HMW:22.4% LMW:10.5%) compared to Ontario (all cases: 4.2% HMW:13.8% LMW:0%). There was also a decrease in the rates of ER visits for asthma in WEA subjects one year after the diagnosis (QC=2.9% ON=3.4%). There was a decrease in the hospitalization rates for asthma after the diagnosis (QC= OA:6.3% WEA:2.9% ON= OA:4.2% WEA:3.4%).

Deliverables: There was a greater reduction in ER visits for asthma in the years after the diagnosis of OA in Quebec than in Ontario, compared to the year preceding the diagnosis of OA mainly in subjects exposed to low-molecular weight agents. Further study is needed to establish if these observations are related to the different type of investigation methods used in each province or to the characteristics of the population studied.

Relevance: The comparison of the health care utilization of workers with WRA between Quebec and Ontario will allow us to identify whether the type of investigation performed has an impact on the reduction of asthma exacerbations following the diagnosis of WRA. The results from this study will be published in academic medical journals pertaining to the areas of occupational health, pulmonary medicine and epidemiology.

4C: Decisional Conflict in Asthma Management

Programme C - Public Health, Ethics, Policy and Society

Authors: **Annick Des Cormiers**, France Légaré, Louis-Philippe Boulet
Institut universitaire de cardiologie et de pneumologie de Québec
Supervisor: Dr. Louis-Philippe Boulet

Objective/Purpose: To evaluate the decisional conflict in asthma management in order to develop shared decision aids.

Methods: This study was based on the Ottawa Decision Support Framework. A decisional conflict scale, a knowledge questionnaire, and an asthma control questionnaire were completed by 50 subjects. They were all aged between 18 and 65 years and had a previous diagnosis of mild to moderate asthma. Decisional conflict was considered meaningful with a score greater than 2/5.

Findings: The proportion of subjects with a meaningful decisional conflict score (DCS) was 70% (median DCS 2.28/5). Also, subjects who were not followed by a physician for their asthma had a significantly higher DCS than those who were followed (p-value = 0.03). A significantly negative correlation was found between patient's knowledge and decisional conflict score.

Deliverables: The results obtained from this innovative study will be published in peer-reviewed journals and will be presented at national and international conferences. Those findings will guide the development of shared-decision aids.

Relevance: This study shows that there are a large proportion of adults that are uncomfortable with decisions related to the control of their asthma, suggesting the need to create aids to help reduce decisional conflict.

This research shows that a discomfort exists in patients with asthma regarding the management of their disease. As a result of this finding, we will develop shared decision aids. These new tools will create discussion between physicians and their patients that will help reduce the decisional conflict. The support of a shared-decision aid may improve the compliance of patients to their treatment and thus improve their asthma control and quality of life.

With various publications, conferences, and support from the Laval University Chair in Knowledge Transfer in Respiratory and Cardiovascular Health, our findings will be communicated to decision-makers/end-users. Furthermore, the shared-decision aids will directly be distributed to physicians and educators to be used with their patients.

5C: Aboriginal Children with Respiratory Problems and Their Parents: Health Inequities

Programme C - Public Health, Ethics, Policy and Society

Authors: **Roxanne Blood**; Amber Ward, Sharon Anderson, Miriam Stewart, Malcolm King, Jeff Masuda Jeff, Heather Castleden, Nicole Letourneau, Lisa Bourque Bearskin, Shawna McGhan
University of Alberta
Supervisors: Dr. Miriam Stewart and Dr. Malcolm King

Objective/Purpose: Aboriginal children and their parents visit emergency rooms more frequently for asthma, have more physician visits, and their asthma is poorly controlled. In spite of higher rates of respiratory illness and health care utilization for Aboriginal children, factors underpinning inequitable health outcomes are poorly understood. One objective of the AllerGen funded study, *Engaging Aboriginal families affected by allergies and asthma in support-education program development* was to assess the challenges experienced by Aboriginal children with asthma and allergies and their parents. The objective of this poster presentation is to report on the health inequities experienced by Aboriginal children and their parents as they attempted to manage asthma allergies.

Methods: This exploratory study employed a participatory research design, as well as qualitative methods to enhance understanding of sensitive issues and meanings, perceptions, beliefs, values, and behaviors of vulnerable groups (Boffa, King, McMullin, & Long, 2011). Aboriginal research assistants conducted individual interviews with 46 Aboriginal children and adolescents who had asthma and/or allergies (26 First Nations, 19 Metis,1 Inuit) and 51 parents or guardians of these children. Sixteen adolescents and 25 parents/ guardians participated in follow-up group interviews.

Findings: Health and health care inequities experienced by affected children and by their parents emerged as a significant problem in diagnosis and management of asthma and allergies. Participants reported inadequate educational resources, environmental vulnerability, social and cultural pressures, exclusion, isolation, stigma, blame, and major support deficits. They also described barriers to access of health services, inadequate health care, disrespectful treatment and discrimination by health service providers, and deficient health care insurance.

Deliverables: These children, adolescents and parents recommended culturally appropriate support and education programs delivered by Aboriginal peers and health professionals.

Relevance: Prior to our research, studies have not focused on health inequities and health care inequities from the perspective of Aboriginal children and adolescents affected by asthma and/or allergies and their parents. Our study revealed that social support deficiencies, income gaps, institutional barriers, and policy limitations were significant factors influencing Aboriginal children's and parents' health behaviors and use of health services.

6C: Use of Complementary and Alternative Therapies in Patients with Asthma: Preliminary Results from a Prospective Study

Programme B - Diagnostics and Therapeutics

Authors: **Mohsen Sadatsafavi**[2], Roxanne Rousseau[1], Larry D. Lynd[2], Carlo A. Marra[2], Wan C. Tan[1], Mark J. FitzGerald[1]
[1]Faculty of Medicine, University of British Columbia, Vancouver
[2]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver
Supervisor: Dr. Carlo Marra

Objectives/Purpose: Asthma patients are increasingly using complementary and alternative therapies (CATs) in the management of their asthma; however, the extent of their use is poorly documented. As part of an ongoing study, we prospectively documented patient reported use of CATs.

Methods: We recruited subjects 1-85 years from two geographic locations in BC, using random digit dialing. Inclusion criteria were a physician diagnosis of asthma and use of at least one asthma-related health care resource in the past five years. At baseline, we collected information on demographic characteristics, use of conventional and alternative therapies, and performed lung function test. We categorized each person based on the level of asthma control (defined per GINA 2006 guidelines including lung function results). We compared the users and non-users of CATs with regard to demographic information and level of asthma control.

Findings: For 280 participants (99% of recruited sample) the data on the use of CATs was complete (mean age 43.6, ±. 20.4, 60.5% female). A total of 75 subjects reported the use of any CAT (26.8%). The most common forms of CATs were breathing exercises and herbal medicines (11.1% each), followed by dieting (5.7%). Homeopathy was the least common form of CAT (1.8%). Among patients reporting the use of CAT compared to non-CAT users, there were no significant differences with regard to age (p=0.44) and gender (p=0.33). There was a significant inverse association between use of CAT and asthma control: among the users of CAT, asthma was fully controlled in 16.9% while in non-users it was fully controlled in 30.1% (p=0.03), according to GINA guidelines.

Deliverables: In our randomly selected sample of asthma patients, asthma control was poor and the use of CAT was high. The inverse relationship between the use of CAT and asthma control could be due to: the complementary role of CAT among patients whose asthma is difficult to control despite medications, a substitute role among patients who are not willing to adhere to conventional therapies for their asthma, or even a potential causal role of some CATs in worsening asthma.

Relevance: Given the high prevalence of CATs in patients with asthma, further studies are required to understand their impact on asthma symptoms, asthma control, and asthma-related resource use. Patients with asthma must be aware that CATs should not interfere with their conventional management and medication use.

7C: The Prevalence of Food Allergy among Aboriginals in Canada

Programme C - Public Health, Ethics, Policy and Society

Authors: **Lianne Soller**, Megan Knoll, Moshe Ben-Shoshan, Daniel Harrington, Lawrence Joseph, Yvan St-Pierre, Sebastien La Vieille, Kathi Wilson, Susan Elliott (McGill, Toronto, and Waterloo Universities, and Health Canada)
Supervisor: Dr. Ann Clarke, McGill University

Objective/Purpose: To estimate the prevalence of food allergy among Aboriginals (First Nations, Métis or Inuit) from the SPAACE study (Surveying the Prevalence of food Allergy in All Canadian Environments).

Methods: We performed a nationwide, cross-sectional telephone survey of all Canadian provinces and territories. Census Canada 2006 data was used to identify postal codes containing a high proportion of Aboriginals. Telephone numbers were randomly selected within these areas. In Nunavut, where addresses were unavailable, the entire population of telephone numbers was surveyed. The household respondent was queried on whether any household member had a food allergy. Prevalence estimates and 95% Confidence Intervals were calculated for each food allergen among individuals reporting Aboriginal status.

Findings: Out of 12,747 households contacted, 6,403 responded (50.2% response rate, representing 15,043 individuals), of which 2,264 (1,336 adults and 928 children) reported Aboriginal status (15.1% of individuals). Among Aboriginal children, 3.95% (2.79, 5.41) reported at least one food allergy. The prevalence of peanut allergy was 1.19% (0.59, 2.11), tree nut, 0.65% (0.24, 1.4), fish, 0.75% (0.3, 1.55), shellfish, 0.22% (0.03, 0.78), sesame, 0% (0, 0.4), milk, 0.54% (0.18, 1.25), egg, 0.65% (0.24, 1.4), wheat, 0.11% (0, 0.6), and soy, 0.22% (0.03, 0.78). In Aboriginal adults, 5.56% (4.40, 6.93) reported at least one food allergy. The prevalence of peanut allergy was 0.82% (0.41, 1.47), tree nut, 0.52% (0.21, 1.08), fish, 0.75% (0.36, 1.37), shellfish, 1.42% (0.86, 2.21), sesame, 0.07% (0, 0.42), milk, 0.45% (0.16, 0.97), egg, 0.37% (0.12, 0.87), wheat, 0.15% (0.02, 0.54), and soy, 0% (0, 0.28).

Deliverables: Our study suggests that the self-reported prevalence of food allergies is lower in the Aboriginal population as compared with a representative sample of the general Canadian population from our previous SCAAALAR project. The overall prevalence of food allergy among children in SCAAALAR exceeded that among Aboriginal children by 4.3% (2.6, 6.0); for tree nut, the difference was 1.1% (0.4, 1.8); for milk, 1.7% (0.9, 2.5); for egg, 0.7% (0.0, 1.4); and for soy, 0.10% (0.28, 0.48). The overall prevalence of food allergy among adults in SCAAALAR exceeded that among Aboriginal adults by 2.7% (1.3, 4.1); for tree nut, the difference was 0.6% (0.2, 1.0); for milk, 1.7% (1.2, 2.2), and for wheat, 0.71% (0.42, 1.00). Although there was a trend for other foods to be less prevalent among Aboriginals, except for fish, which was more prevalent, wide confidence intervals precluded definitive conclusions.

Relevance: The lower prevalence of self-reported food allergy in the Aboriginal population may be attributable to several factors: genetics, differences in dietary habits and the environment, as well as inequities in access to health care, education and information about food allergy.

8C: The Work-related Asthma Screening Questionnaire (long-version) (WRASQ(L)): Reliability & Effect on Provider Behaviour

Programme C - Public Health, Ethics, Policy and Society

Authors: **Katie Killorn**[1,2], S. Dostaler[1], P.A. Groome[2], M.D. Lougheed[1,2]
[1]Asthma Research Unit, Kingston General Hospital, ON, Canada; [2]Department of Community Health & Epidemiology, Queen's University, Kingston, ON, Canada.
Supervisors: Dr. Diane. Lougheed and Dr. Patti Groome

Objective/Purpose: Work-related asthma (WRA) is under-recognized and early detection is associated with improved health outcomes. The WRASQ(L) is a 14-item questionnaire based on four concepts (occupation, the relationship between asthma symptoms and work, inhalation exposures and management of personal exposure). The objectives of this study are to assess whether the WRASQ(L) provides additional and reliable information on a patient's risk of WRA, beyond what is collected in standard practice, and whether or not the WRASQ(L) responses prompt appropriate care provider behavior, in terms of clinical practice guidelines for the assessment of suspected WRA. It is hypothesized that this screening tool will provide greater opportunity to identify patients at risk of WRA and prompt care providers to initiate further investigation(s). Preliminary data will be presented since data collection is ongoing.

Methods: This is a multi-site cross-sectional study assessing detection of WRA in Ontario Primary Care Asthma Program (PCAP) sites assigned an enhanced WRA screening tool (WRASQ(L)). Usual care for asthma patients seen by PCAP asthma educators involves completing the Ontario Lung Association Asthma Care Map (ACM) (which includes seven WRA screening items, also found on the WRASQ(L)). Consent to participate in this study prompts care providers to record details related to referral and testing, if applicable and involves completing a WRASQ(L) at each subsequent visit until March 2012 for patients. Subject responses to the WRA screening items on the ACM will be compared to responses from the WRASQ(L) to assess whether the additional WRASQ(L) items were successful at detecting patients at risk of WRA that may not have been detected with the ACM. The influence of WRASQ(L) responses on care provider behavior (*e.g.* suggesting a specialist referral for those patients with suspected WRA) will be assessed by testing the relationship of WRASQ(L) response patterns to those actions. Individual WRASQ(L) items will also be evaluated for test-retest reliability using Cohen's Kappa.

Findings: Preliminary results include a descriptive analysis of 15 WRASQ(L)s completed by 15 adults (male: 5 female: 10) with asthma, from participating PCAP sites. The mean age of participants was 45.9 years (SD 11.5; range 24-64). Nine participants were employed full or part-time.

Deliverables: The project will provide information regarding the clinical utility of the WRASQ(L) in primary care, to identify patients at risk of WRA and promote further investigation by the care provider.

Relevance: Considering the benefits of early detection, this screening tool may lead to improved health outcomes for patients by expediting diagnoses and increasing the recognition of WRA.

WRASQ(L) Concepts	
Relationship: asthma symptoms	**n**
Start at work	1
Start in the days of a spill or fire at work	0
Worsen at work	4
Worsen on your first day back to work	3
Worsen during the work day	4
Worsen at home after work	2
Worsen throughout the workweek	1
Less on days off work and/or holidays	2
Number of exposures at work	**mean**
Current	4.80 (SD 3.97, range 0-14)
Past	6.07 (SD 4.92, range 0-15)
Avoidance	**n**
Use of protective measures	5

9C: A Novel Way of Preventing and Managing Allergic Disease and Asthma: Real-time Index-based Monitoring of Environmental Factors

Programme C - Public Health, Ethics, Policy and Society

Authors: **Natalia Mykhaylova**, Jeff Brook
SOCAAR, Dept. of Chemical Engineering and Applied Chemistry, University of Toronto
Supervisor: Dr. Greg Evans

Objective/Purpose: The exposure to air pollutant mixtures is a well-known risk factor for inducing and increasing the severity of upper airway allergic disease and asthma (ADA). Air pollutants most commonly linked to these disease outcomes include ozone (O_3), nitrogen dioxide (NO_2) and fine particulate matter (PM). Volatile organic compound (VOC) mixtures have been suggested as surrogates for allergen exposure. The presence of mixtures of these pollutants, even at low concentrations, has been associated with direct impacts on respiratory disease development as well as indirect impacts on ADA-inducing potential of many aeroallergens. For real-time detection and monitoring of ADA-associated pollutant levels and sources, sensor arrays are an optimum choice because of versatility and aptitude for tracking composite multi-pollutant exposure indices. To address this opportunity, a sensor array-based system for tracking Composite Allergy-associated Pollutant Index (CAPI) is being developed and tested.

Methods: Different versions of CAPI instrument prototype have been designed and created for three applications: indoor, outdoor and portable/wearable. Each prototype consists of an array of commercially available sensor technologies: an ionization fine PM sensor, chemiresistive n-type MOS total VOC sensor, and chemiresistive p-type MOS sensors for NO_2 and O_3. Temperature and humidity sensors were included for data normalization. CAPI was derived using measured levels of the pollutants and factors associated with the degree of their contribution towards health impact. The prototype's response has been calibrated against accepted standard measuring techniques: API Photometric Ozone Analyzer, API Chemiluminescent NO_X Analyzer and TSI Aerodynamic Particle Sizer Spectrometer. Level of pollutant exposure and corresponding CAPI range has been characterized in four locations in Toronto: a high-rise residential building overlooking a busy highway, an academic building overlooking a medium-size road, an indoor bus stop and an underground subway station.

Findings: In preliminary tests, the response of CAPI prototypes was shown to be sensitive enough to detect ambient levels of pollutants: below 5ppm level for total VOCs, below 100 counts per cm^3 for fine PM, below 5ppb for O_3, and below 15ppb for NO_2. In particular, roadside residential and academic buildings showed high levels of NO_2 and O_3, while the indoor bus stop and subway station showed high levels of PM. The pollutant levels also exhibited large variation both temporally and spatially, indicating the importance of real-time monitoring of these pollutants as well as the benefits that a portable CAPI instruments, or dense CAPI instrument networks, could hold for helping patients manage their asthma.

Deliverables and Relevance: This proposed air pollutant mixture monitoring instrument, as well as CAP index, represent unique, flexible, and low cost solutions for managing ADA and could improve the quality of life of allergy sufferers by providing feedback on the air contaminants they are exposed to, possible sources and mitigation strategies. It could also help identify populations at risk of development of ADA and promote more targeted and effective air pollutant policy interventions.

10C: Who's Willing to Trade in the Family Dog? Socioeconomic Status (SES) Predicts Differential Avoidance of Asthma Triggers

Programme C - Public Health, Ethics, Policy and Society

Authors: **Kathleen Nelligan**[1], Richard Foty Msc[1], David Steib MD[2], Teresa To PhD[1], Sharon Dell MD[1]
[1]Hospital for Sick Children, Toronto, ON; [2]Health Canada, Ottawa, ON
Supervisor: Dr. Sharon Dell

Objective/Purpose: Guidelines state avoidance of asthma triggers is critical to asthma management. Studies suggest that SES predicts differential trigger exposure and low SES predicts poorer asthma control. We sought to determine whether SES predicts trigger avoidance.

Methods: Data from the Toronto Child Health Evaluation Questionnaire (T-CHEQ), a population-based cross-sectional survey, was used. Doctor-diagnosed asthma, household income, allergen and irritant exposures, and modifications to home environment were defined by parental report. A nested case-control survey collected a complete history of home allergen exposures. SES was defined by income adequacy (IA; income adjusted for household size). Multivariate logistic regression was used to determine the association between IA and avoidance of asthma triggers. Statistics were conducted using SAS-9.2.

Findings: 5619 children participated; 15% of children had doctor-diagnosed asthma; 36.90% were from high IA homes, 22.79% from high-mid, 22.58% from low-mid and 17.74% from low. Among children with asthma, low IA was associated with current cockroach (OR 160.61, 95%CI 22.32-1155.74) and tobacco-smoke (OR 1.96, 95%CI 1.01-3.79) exposure but was protective against having dogs (OR 0.19, 95%CI 0.07-0.57), cats (OR 0.41, 95%CI 0.17-0.97) or furry pets (OR 0.27, 95%CI 0.08-0.91). Among children with allergies or asthma low IA was associated with altering pillows (OR 1.48, 95%CI 1.00-2.20) and bedding (OR 2.16, 95%CI 1.42-3.29), and removing pets (nested sample) (OR 5.93, 95%CI 1.25-28.13). Removing pets remained significant when limited to children with asthma (OR 8.39, 95%CI 1.11-63.67).

Deliverables: The results provide evidence for differential exposure to asthma triggers in a Canadian city and indicate that low IA families take steps to reduce exposure for their children. The findings also suggest that high IA families may be less likely to comply with guidelines on asthma trigger avoidance.

Relevance: The results of this investigation suggest that differential exposure to asthma triggers might explain some variability in asthma control between income groups. Although low IA families reported attempts to modify exposure to certain triggers, some exposures may be difficult to ameliorate (i.e. cockroach). This might provide a potential avenue for use of biologics such as monoclonal antibodies for the treatment of atopic asthma.

11C: Asthma Control in a Random Sample of Canadian Asthma Patients

Programme C - Public Health, Ethics, Policy and Society

Authors: **Mohsen Sadatsafavi**[2], Roxanne Rousseau[1], Larry D Lynd[2], Carlo A Marra[2], Wan C. Tan[1], Mark J FitzGerald[1]

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Supervisor: Dr. Carlo Marra

Objectives/Purpose: The reported levels of asthma control have not always been population-based and may therefore be severely biased by the sampling method.

Methods: We recruited subjects, 1-85 years, in British Columbia (BC) using random digit dialing. Included subjects had a physician diagnosis of asthma in the past five years. At baseline, we collected information on the demographic and socioeconomic characteristics, did spirometry, and determined the level of asthma control according to the Global Initiative for Asthma (GINA). We performed a proportional-odds ordinal logistic regression analysis with asthma control level (controlled=2, partially controlled=1, uncontrolled=0) as the dependent variable among the sub-sample of adolescents and adults for whom socio-economic data were available.

Findings: Control level could be assessed for 272 subjects (97% of sample, mean age 43.9 years, s.d. 21.0, 59.3% female). Of these, 67 (24.6%) were controlled, 109 (40.1%) were partially controlled, and 96 (35.3%) were uncontrolled. The **Table** shows the results of the regression analysis among the adolescent and adult asthmatics for whom baseline data was complete (n=232). The only two factors associated with the level of control were gender (p=0.05) and whether or not the patient was born in Canada (p=0.03). However, there was no association between these two factors (p=0.78 for gender, p=0.43 for place of birth) and asthma control among children (n=33).

Table: Results of the multivariate ordinal regression analysis of potential predictors of asthma control among the adolescent and adult (n=239)

Covariate	Odds Ratio*	95% Confidence Interval
Age	0.99	0.97 – 1.10
Gender (female vs. male)	0.60	0.36 – 1.00
Canadian-born	1.78	1.04 – 3.06
Job status (employed vs. unemployed)	1.20	0.63 – 2.31
Income range (cutoff 40K/year)	0.89	0.52 – 1.52
Receiving extended insurance	1.40	0.80 – 2.47
Years since diagnosis of asthma (more vs. less than 10 years ago)	0.99	0.54 – 1.80

*Odds ratios are for successive levels of control (controlled vs. partially controlled, and partially controlled vs. uncontrolled) in an ordinal regression model. The assumption of the proportionality of odds was not rejected p=0.60)

Deliverables: Our results, obtained from a random sample in BC, showed a substantial lack of asthma control. There was no difference in the level of asthma control between sexes in children. However, among adolescents and adults, females had greater risk of poorer control. Our results may help policy makers with regard to strategies for targeting populations to achieve better asthma control.

Relevance: It is well known that control can be achieved in the vast majority of asthma patients, and that lack of control is a significant driver of costs. Our results therefore, underline a dire need in better management of asthma in the province, with a potentially high return on investment in terms of reduced asthma costs and improved quality of life among patients.

12C: Determinants of Purchasing Behaviors of Allergy Affected Canadian Consumers: A Mixed Methods Approach

Programme C - Public Health, Ethics, Policy and Society

Authors: **Bonnie YL. Chow**[1], S. J. Elliott[2], M. D.W. Harrington[3], Ben-Shoshan[4], A. Clarke[4], J. Fragapane[4], L. Soller[4], S. B. Godefroy[5]
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Supervisor: Dr. Susan J. Elliott

Objective/Purpose: The purpose of this research is to combine new qualitative data with existing quantitative results, in order to provide an in-depth understanding of the determinants of consumption behaviors and perceptions of allergen labels among affected Canadians.

Methods: This research drew upon a national Canadian survey (SCAAALAR) that asked respondents to self-report purchasing behaviours and perceptions of Canadian allergen labels (n=1380). The new qualitative portion involved in-depth interviews with families (n=12) with anaphylactic allergies to peanut, tree nut and/or sesame. Interviews were conducted in the store setting during a regular shopping trip and covered topics related to grocery shopping, use of and perceptions of allergen labels. Qualitative thematic analyses were facilitated by NVivo.

Findings: Quantitative results indicate 20% of Canadian families are directly affected by food allergies, while 31.4% are indirectly affected, *i.e.*, family does not have an allergic member, but has responsibilities preparing food for an allergy-controlled environment. Quantitative analyses suggest that current Canadian allergen labels are not as effective as expected, since affected consumers reported not always heeding precautionary statements. Allergic families were found to be less diligent than indirectly affected families, and also less likely to find precautionary statements helpful. Through qualitative interviews, it was found that prior experience, not allergen information, is the primary factor guiding purchasing decisions. Even though precautionary statements were found to be easy to understand, terminology, font sizes, and contrast issues were reported to be key areas for improvement.

Deliverables: This research provides a preliminary understanding of the differences in individuals' social constructions of risk, which ultimately shape purchasing and consumption behaviors. This research will be used to inform future research to understand more fully the social construction of food allergy risk.

Relevance: This study is one of the first in the literature to qualitatively explore attitudes toward allergen label use in Canada, which is critical for guiding the next phase of policy change related to precautionary statement use. Through the research team's partnership with Health Canada and regional allergy support groups, results and/or input will be disseminated and received by decision makers and end-users.

13C: The Impact of Recruitment Methods on CHILD Study Enrollment

Programme C - Public Health, Ethics, Policy and Society

Authors: **Jing Luo**[1,2], Chooniedass, R[1,2], Kozyrskyj AL[3,4,] Ramsey CD[4,5], Becker, AB[1,2]
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Supervisor: Dr. Allan B. Becker

Objective/Purpose: To determine the effectiveness of different recruitment methods to enroll study participants in an observational pregnancy cohort study.

Hypothesis: Advertising using different forms of media (television, radio, and newspaper) will yield the highest rate of recruitment compared to all other methods and inclusion of an informational survey for an incentive will enhance enrollment.

Methods: The Canadian Healthy Infant Longitudinal Development (CHILD) study is a national study recruiting 5,000 pregnant women. Winnipeg is one of the four recruiting sites. Various recruitment strategies were used to recruit pregnant women before 32 weeks gestation. Five main recruitment methods were initially implemented: direct advertising, community events, physician referral, prenatal classes and personal contact. A one page environmental and health survey which included an incentive was later implemented at the various recruitment sites.

Findings: Over three years, Winnipeg screened 3309 potential participants, and enrolled 1095 families. Recruitment rates utilizing the different strategies were as follows: physician referral (488/1095,44.6%), community events (263/1095,24.0%), personal contact (study staff and participants) (264/1095,24.1%), prenatal class (48/1095,4.4%), and direct advertising (32/1095,2.9%). 23.4% (164/701) of participants were enrolled in the study after completing the environmental and health survey. 71(6.4%) families were referred by current study participants and were successfully enrolled into the study.

Deliverables: Passive advertising (poster and brochure) yielded the lowest number of recruits. Physician referral remains the most effective method to enroll study participants.

Relevance: Determining the most effective recruitment methods will facilitate future recruitment strategies.

Limitation: Unfortunately of 1019 physicians approached to participate, only 30 physicians are actively recruiting. Concentrating on physician involvement could significantly increase enrollment.

14C Mothers' Potential for Self blame in Reaction to a Genetic Association Study

Programme C - Public Health, Ethics, Policy and Society

Authors: **Alicia Pawlowski**[1], I. Poukhovski-Sheremetyev[1], N. Letourneau[2], K. Hegadoren[1], D. Daley[3], J. Austin[3], A. L. Kozyrskyj[1,4]
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Supervisor: Dr. Anita L. Kozyrskyj

Objective/Purpose: Much debate surrounds the potential tradeoff between genetic risk awareness and anxiety. Yet few investigations have focused on the mother, who through her unique position has historically been held responsible for the afflictions of her children. Recent evidence that maternal stress in early life may lead to the development of childhood asthma,provides an opportunity to elucidate women's thoughts and beliefs in this area.

Methods: 208 of 470 mothers emailed from the Canadian SAGE and MOMS studies, responded to a web-based survey on their lived experience of and beliefs about PPD and childhood asthma (a participation rate of 44.25%). Mothers were asked to assess the degree of self-blame they would assume for their child's asthma in reaction to 'hypothetical' genetic and non-genetic research scenarios which revealed a connection between PPD and childhood asthma. Women also reported if the research information would prompt them to take preventive measures in future pregnancies. Multiple logistic regression was used to determine the association between women's personal history of and beliefs about PPD and child asthma, general opinion about genetics research or testing, and elicited self-blame/preventative action for childhood asthma.

Findings: While most women would not blame themselves for their child's asthma, self-blame when present was directly influenced by personal experience (having had PPD and a child with asthma), and a belief that genetic testing was harmful (OR, 2.6; 95 % CI, 1.3-5.4, $p < 0.01$). In mothers with asthmatic children, those with a history of PPD were *more* likely to blame themselves than those without in the genetics scenario (OR, 4.8; 95% CI, 1.6-14.1, $p < 0.005$). It was also observed that women with a personal experience of PPD and childhood asthma, who might benefit from presented findings, were least likely to report an intention to take preventative measures in the future.

Deliverables: Relevant personal history determines self-blame as a reaction to research information. Consistent with previous research, learning about genetic risk does not seem to motivate key demographics to change their behavior.

Relevance: This research presents an empirically-based evaluation of factors which could sensitize mothers to epidemiologic and genetic research findings, linking maternal postnatal stress and depression to childhood asthma. It is key to practitioner and researcher understanding of the impact of widespread epidemiologic and genetic risk information on both target individuals as well as the general public.

15C: Use of Social Media in the CHILD Study

Programme C - Public Health, Ethics, Policy and Society

Authors: **Claire Unruh**[1,2], Chooniedass, R[1,2], Kozyrskyj AL[3,4,] Ramsey CD[4,5,] Becker, AB[1,2]
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Supervisor: Dr. Allan B. Becker

Objectives/Purpose: With hundreds of millions of users and a straightforward means of disseminating information, social media allows for widespread accessibility to shared materials. Researchers are now able to use social media to connect with other researchers, communities and even their own study participants.

Hypothesis: Awareness and subsequently recruitment for the CHILD (Canadian Healthy Infant Longitudinal Development) Study will increase by communication via social media.

Methods: The CHILD study is a national study recruiting 5,000 pregnant women and following their newborns until five years of age, to assess the impact of the environment on childhood asthma and allergic disease. A mass email was sent to 900 CHILD participants enrolled at the Winnipeg site, notifying them of the creation of a Facebook page for the CHILD study. We undertook an assessment of the uptake of social media related to the CHILD study (defined as Facebook and email). A total reach included anyone who had seen information from the site. Direct communication was defined as anyone who became a member of our Facebook page. Indirect communication was defined as anyone who was connected with a member and was able to see the information posted.

Findings: Within 4 months, the popularity of CHILD's Facebook page grew from 9 to 88 members and had a total reach of 236 people. Through the availability of social media, individuals from 19 cities in 12 countries have seen information on the CHILD study. The inter-connectivity of members on Facebook showed that within this short time period, the CHILD study was able to reach 22,213 people globally through direct or indirect communication.

Deliverables: Social media provides the opportunity to advertise the CHILD study and enhance recruitment, connect study participants and researchers in a simple fashion and help CHILD researchers find other collaborators.

Relevance: Through the connectedness and information sharing of an online community, such as Facebook, a longitudinal study like CHILD will demonstrate how use of social media can help to retain study members for a minimum of five years. Our social media initiative will also evaluate the extent to which the CHILD research team is able to connect with other health groups, organizations and researchers to ensure a continuing flow of information.

IV. NON-ADJUDICATED POSTERS

#	Trainee		Institution	Supervisor(s)	Abstract Title
1NA (B)	**Omana,** Vanessa	MSc	Queens University	Anne K. Ellis	Optimizing RNA Extraction and Reverse Transcription Techniques for Q-PCR Analysis of Molecular Biomarkers of Eosinophilopoiesis in Umbilical Cord Blood
2NA (C)	**Ben-Shoshan,** Moshe	PDF	McGill University	Ann Clarke	Anaphylaxis in Children Treated in the Montreal Children's Hospital: Rate, Clinical, Characteristics, Triggers and Management

1NA: Optimizing RNA Extraction and Reverse Transcription Techniques for Q-PCR Analysis of Molecular Biomarkers of Eosinophilopoiesis in Umbilical Cord Blood

Programme B - Diagnostics and Therapeutics

Author(s): **Vanessa Omana** (BSc), Jenny Thiele (MSc) and Anne K Ellis (MD, MSc, FRCPC), Queen's University
Supervisor: Dr. Anne K. Ellis

Objective/Purpose: We have previously identified molecular biomarkers of eosinophil-lineage commitment in cord blood that can serve as surrogates for the activity of the CD34+ progenitor during IL-5 induced eosinophilopoesis (GATA-1, MBP1 and IL5R isoforms). Whether or not these biomarkers are predictive of future atopic risk in the newborn is yet to be fully established, as the GATA-1 assay in particular is challenging to replicate across different qPCR platforms. We sought to optimize the protocol in order to allow for easier determination of differences in the kinetic expression patterns of these biomarkers in infants at high versus lower atopic risk, in a manner that can be utilized in several labs. In this study, we will compare three RNA extraction protocols followed by three reverse-transcription assays to optimize our experimental yield. In addition, we will examine the kinetic mRNA expression of GATA-1 in response to IL-5 stimulation to investigate its potential as a putative biomarker of future atopy.

Methods: Consent was given by expectant mothers and umbilical cord blood was collected after delivery. Samples were assigned high or low atopic risk, depending on maternal atopic status. Mononuclear cells (MNCs) were isolated using an Accuprep® gradient and stored in liquid nitrogen. Upon thawing, the MNCs were incubated in McCoy's 3+ media for two hours. The non-adherent monocuclear cells (NAMNCs) were seeded at a concentration of $1x10^6$ cells/ml and stimulated with recombinant interleukin 5 (IL-5, 1ng/mL) for 0, 24, 48, and 72 hours. At these time points cells were collected into RNAprotect® cell reagent and frozen at -80°C to allow for simultaneous future analysis. RNA was isolated using three different RNA protocols: Froggabio Total RNA Mini Kit, Qiagen RNEasy Plus Mini Kit, and phenol-chlorophorm RNA extraction. Our initial analyses will focus on comparing the RNA yield from all three protocols. The samples will then be reverse-transcribed. Ultimately, this study will focus on using q-PCR analyses to compare kinetic expression patterns of GATA-1 mRNA of NAMNCs from infants with high and low atopic risk.

Findings and Deliverables: In Progress

Relevance: Given the increasing prevalence of atopic diseases, the determination of novel molecular biomarkers predictive of future allergy in the newborn would not only allow for the development of a diagnostic test, but could also provide insight towards new anti-allergic therapeutics. This project will determine the optimal procedure for RNA isolation and reverse-transcription which will lead to improved RNA/cDNA yields and thus an enhanced protocol of qPCR analysis for these biomarkers that can be applied in multiple laboratories.

2NA: Anaphylaxis in Children Treated in the Montreal Children's Hospital: Rate, Clinical Characteristics, Triggers and Management

Programme C - Public Health, Ethics, Policy and Society

Authors: **Moshe Ben-Shoshan**, MD, MSc[1] , Sebastian La Vieille, MD[2], Harley Eisman, MD[3], Reza Alizadehfar, MD[1], Emma Perkins, BSc[4], Lawrence Joseph, PhD[4 5] Ann Clarke, MD, MSc[4 6]
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Supervisor: Dr. Ann Clarke

Objective/Purpose: To determine the incidence of and characterize anaphylactic reactions in a pediatric emergency department (ED).

Methods: As part of the Cross-Canada Anaphylaxis REgistry (C-CARE), members of the ED in the Montreal Children's Hospital were asked to recruit children with anaphylaxis (defined as involvement of two organ systems and/or drop of blood pressure in response to a potential allergen). After parents consented, the treating physician reported on the clinical characteristics, potential triggers and management of the anaphylactic reaction. In addition, the charts of all patients visiting the ED were reviewed daily to identify anaphylactic cases that were not recruited prospectively.

Findings: Among 47,959 ED visits between April 26 and December 5 2011, 41 met the definition of anaphylaxis, *i.e.*, 0.085% (95% CI, 0. 062%, 0. 117%). The median age was 4.1 years (IQR: 2.2, 12.6) and 53.6% (37.6%, 69.0%) were males. Food was responsible for 80.5% (64.6%, 90.6%) of reactions with peanut being the major culprit [36.4% (21.0%, 54.9%)]. Insect stings were associated with 12.1% (4.6%, 27.0%) of reactions. Other exposure (cleaning spray) was reported in one case. In 4.9% (0.9%, 17.8%) of cases, the trigger was unknown. The majority of reactions occurred at home (68.3%, 51.8%, 81.4%). Prior to arrival in the ED, epinephrine was administered to 24.4% (12.9%, 40.6%), antihistamines to 19.5% (9.4%, 35.4%), and in 53.7% (37.6%, 69.0%), no treatment was given. In the ED, 56.1% (39.9%, 71.2%) of cases received epinephrine and antihistamines.

In 74.4% (57.6%, 86.4%), epinephrine was prescribed at discharge from the ED and in 23.1% (11.7%, 39.7%) it was not prescribed as the patient already had an auto-injector. Seventy-seven percent (59.5%, 89.0%) were referred to an allergist and 20% (9.1%, 37.5%) were already followed. In 58.5% (42.2%, 73.3%), there was allergy to a known allergen and in 19.5% (9.4%, 35.4%), there was pre-existent asthma. Among all anaphylactic reactions, 14.6% (6.1%, 29.9%) were severe (defined as the occurrence of hypoxia, cyanosis, circulatory collapse, incontinence or neurological symptoms). Of these, only 33.3% (6.0%, 75.9%) received epinephrine prior to arrival in the ED, while 83.3% (36.5%, 99.1%) received epinephrine in the ED. Using a multivariate logistic regression, asthma was the only factor associated with reaction severity (OR= 10.3, 1.5, 974.3).

Deliverables: Our results indicate that anaphylaxis is a substantial health problem that is relatively undertreated outside of the hospital.

Relevance: This is the first prospective study on anaphylaxis. We aim to expand the current study to include other health centers as well as the emergency medical services across Quebec to improve knowledge gaps and management of anaphylaxis.

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