Summer 2013

AllerGen

Innovation from cell to society

Success Stories

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Networks of Centres of Excellence of Canada | Réseaux de centres d'excellence du Canada

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Making a Difference
From cell to society

AllerGen NCE Inc. (AllerGen), the Allergy, Genes and Environment Network — one of Canada's Networks of Centres of Excellence (NCE) — is pleased to present its sixth issue of *Success Stories*, showcasing research accomplishments of leading Canadian allergy, asthma, anaphylaxis, genetics, environment and education researchers, their students, and partner and patient stakeholder organizations.

Since its inception in 2005, AllerGen has supported innovative research and development, capacity building activities, stakeholder engagement and partnerships that foster research commercialization, social innovation and knowledge mobilization, enabling Canadians to better prevent, manage and treat allergy, asthma, anaphylaxis and related immune diseases.

Through its national network of allergy, asthma and immune disease experts, AllerGen engages 270 Network Investigators and collaborators, 474 students, junior professionals, research associates and technicians, and works closely with over 192 partner organizations across academic, industry and government sectors.

In this issue of *Success Stories*, we share the highlights of five AllerGen-supported research projects. Feature stories include:

- how diesel exhaust changes gene function in people with mild asthma;
- why some people are allergic to peanuts and others are not;
- the challenge of measuring indoor exposure to phthalates;
- a new way of thinking about the relationship between the nose and lungs; and
- Canada's 'rising star' in allergy research.

Asthma and allergic diseases are a global public health concern and their prevalence continues to increase worldwide. In Canada, one in three people now lives with allergic disease. The associated economic and healthcare burden of these conditions reaches billions of dollars annually.

Through to 2019, AllerGen will continue to focus its research, commercialization and knowledge mobilization efforts on:

Three Enabling Platforms:

- **Gene-Environment Interactions,**
- **Biomarkers and Bioinformatics,**
- **Patients, Policy and Public Health; and**

Three Legacy Projects:

- **Canadian Healthy Infant Longitudinal Development (CHILD) Study**
  This national birth cohort study has been tracking over 3,000 children since before they were born to shed light on how allergic disease and asthma develop in early childhood.

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- **Clinical Investigator Collaborative (CIC)**
  This multi-centre, Canadian-based Phase II clinical trials group enhances drug discovery for allergic asthma, allergic rhinitis and severe asthma.
- **Canadian Food Allergy Strategic Team (CanFAST)**
  This innovative, multidisciplinary team addresses the critical question: How are the prevalence, perception and experience of food allergy in Canada changing over time? CanFAST research provides families, health care professionals and policy makers with the first Canadian prevalence data, which will help to prevent life-threatening anaphylactic reactions and lay the foundation for a national food allergy strategy.

In sharing these stories, AllerGen aims to decrease the burden that allergy and asthma impose on Canadian productivity and economic growth, contribute to Canadian innovation and commercialization, and improve the quality of life for Canadians living with allergic diseases, asthma, and anaphylaxis.

We hope you enjoy reading this issue of *Success Stories*!

Judah Denburg, MD, FRCP(C), Scientific Director and CEO

Diana Royce, EdD, Managing Director and COO

Researchers agree that the question is no longer whether or not exposure-related epigenomic change — or more simply, the change in gene function due to environmental influences — is at play in asthma and allergies. Evidence shows that it is.

How Diesel Exhaust Changes Our Genes

In airspace and on roads, railways and waterways throughout the world, diesel engines are commonplace — and their use is increasing, particularly in developing countries. No wonder: they are more reliable and fuel efficient than any other internal combustion engine, which translates into savings at the gas pump.

Unfortunately, however, the sooty cloud billowing from the exhaust pipe of a diesel-powered vehicle is more than just a smelly, gritty annoyance. Ultrafine particles contained within diesel exhaust easily pass through the nose and throat and lodge in the lungs, where their effects penetrate right down to the molecular level — even to the level of our very genes — and appear to lay the groundwork for the development of asthma and other diseases.

Dr. Chris Carlsten, Associate Professor of Medicine, University of British Columbia

Research in the relatively new field of epigenetics has shown that while our inherited genes map out the blueprint of how we develop, genes can be modified — switched on or off, dialed up or down — by factors in the environment. Exposure to diesel exhaust, it turns out, is one of those factors.

Diesel Exhaust and the Human Genome

Dr. Chris Carlsten, an AllerGen Principal Investigator and Associate Professor of Medicine at the University of British Columbia (UBC), researches the health effects of air pollution. With AllerGen colleagues Drs Michael Kobor and Scott Tebbutt, and Dr. Stephen Jackson at Nanostring Technologies, Dr. Carlsten is working to understand how diesel exhaust affects asthma and allergies. Toward this end, his team recently conducted a study to better understand how diesel exhaust, through its impact at the genetic level, can provoke asthma exacerbation (or 'asthma attacks').

Researchers agree that the question is no longer whether or not exposure-related epigenomic change — or more simply, the change in gene function due to environmental influences — is at play in asthma and allergies. Evidence shows that it is. The current scientific challenge is to conclusively determine what epigenomic changes occur, and how quickly, upon exposure.

Dr. Carlsten believes that clarifying the mechanics of epigenomic change provoked in asthmatics by exposure to air pollution may help to explain why asthma rates are climbing, and why traditional genetic association studies have been unable, so far, to identify the cause.

Real Life in the Lab

Evidence from previous studies shows that asthmatics exposed to the sooty particles in diesel exhaust suffer exacerbations of their asthma symptoms. But exactly why this happens is poorly understood. Dr. Carsten suspected that epigenomic processes were involved and set out to find them.

With funding from AllerGen NCE, WorkSafeBC and the Vancouver Coastal Health Research Institute, Dr. Carlsten's team designed a study to look for two specific epigenetic processes that they thought might be triggered by exposure to diesel exhaust: DNA methylation and changes to microRNA. In DNA methylation, certain chemicals forming what is known as a 'methyl group' are added to the DNA 'backbone' — the core supporting structure of the well-known DNA double helix. If methylation occurs in a particular region of a gene, the function of the gene may be altered. MicroRNA, on the other hand, are a subset of the ribonucleic acid (RNA) family of molecules that influence genetic function. A change in microRNA can change the ways in which genes are expressed (*i.e.*, the way a gene works).

The researchers recruited a group of asthmatics whose condition was mild enough to be controlled with only bronchodilator medication, and a control group of non-asthmatics, to participate in the study. Subjects were invited to the Air Pollution Exposure Laboratory (APEL) at UBC and asked to ride an exercise bike intermittently over two hours. While they rode, they were exposed to either diluted diesel exhaust — air quality similar to that of a highly polluted city or an underground mine — or filtered, clean air.

In the mild asthmatics, inhaling diluted diesel exhaust triggered a number of immediate effects at the genetic level — effects that lasted over a 30-hour period, with the most significant changes occurring between six and 30 hours following exposure.

Samples of the subjects' blood were taken before the experiment began and at two, six and 30 hours after exposure. The order in which the exposures were scheduled was randomized from subject to subject. Two weeks after the first exposure the experiment was repeated, exposing the subjects to the alternate air quality.

Dr. Carlsten's unique study design makes this research particularly relevant to real life. First, the research protocol mimicked people moving about at a normal, everyday pace while exposed to air of a quality similar to that found in some of the world's larger cities. Second, the blinded, cross-over design, which exposed study participants to both forms of air under conditions that were otherwise exactly the same, precluded undesirable variability in the results.

"This is a powerful design because the only thing that changes is the air quality," said Dr. Carlsten. "Everything else remains precisely the same — the length of exposure, the procedure used, the person involved. Even an individual's genetic background is essentially made irrelevant, because that genetic 'backbone' does not change."

Diesel Exhaust May Suppress Protective Genes

When Dr. Carlsten's team analyzed participants' blood samples, the findings were intriguing. In the mild asthmatics, inhaling diluted diesel exhaust triggered a number of immediate effects at the genetic level — effects that lasted over a 30-hour period,

Subjects were invited to the Air Pollution Exposure Laboratory (APEL) at UBC and asked to ride an exercise bike intermittently over two hours. While they rode, they were exposed to either diluted diesel exhaust or filtered, clean air.

The Air Pollution Exposure Laboratory at UBC

with the most significant changes occurring between six and 30 hours following exposure.

At least one of these effects involved DNA methylation. AllerGen trainee Dr. Francesco Sava studied genes known to produce proteins that protect the body from 'oxidative stress' — a harmful condition that is associated with the development of asthma, caused by, among other things, air pollution. When exposed to diesel exhaust, in some of the study participants these genes became increasingly methylated. Though the changes were not of a magnitude likely to have true biological significance — by design, the study did not intend to produce any change of clinical relevance — similar changes of a larger magnitude could affect the ability of these genes to produce protective proteins that ward off the negative effects of oxidative stress. As the body becomes vulnerable to oxidative stress, exacerbations of asthma occur, and so this new finding may play a role in determining an individual's vulnerability.

Local Research, Global Change

This is the first study to look at the short-term (within hours) epigenetic effects of diesel exhaust — a crucial topic given the global prevalence of diesel engines. The study design was such that none of the changes would have true functional importance, but with the team's discovery that genetic changes can be triggered within mere hours, rather than months, years or decades after exposure, come the inevitable questions: "Can we prevent these changes?" and "What happens with repeated exposures of similar magnitude?"

"There is a need to understand the short-term dynamics of DNA methylation and how quickly things change," stated Dr. Carlsten. "This knowledge is needed before we can assess how significant these changes might be."

Dr. Carlsten hopes that this research will inform future studies and eventually lead to the development of screening, prevention and therapeutic tools for asthma exacerbation. He also believes that research like this contributes in a fundamental way to the long-term creation of a healthier world.

"If you look at the history of air pollution literature over the past 100 years, and then look at the development of public health policies, you can see a very clear relationship between that research and health benefits on a global level," said Dr. Carlsten. "We believe that this study is a small, but very real part of a large body of international work on asthma and air pollution that will translate into public health benefits."

The frightening aspect of peanut allergy is that it can be fatal, with effects that range from mild rashes and hives to life-threatening anaphylactic shock.

Cracking the Case of Peanut Allergy

Why are some people allergic to peanuts and others not?

It's a question that scientists around the world are struggling to answer. What we do know is that peanut allergy is partially influenced by genetic factors — studies of families and twins have shown this. However, exactly how our genes contribute to the development of peanut allergy remains a mystery.

Dr. Andrew Sandford, Associate Professor, University of British Columbia's James Hogg Research Centre

Dr. Catherine Laprise, Professor, Université du Québec à Chicoutimi

The frightening aspect of peanut allergy is that it can be fatal, with effects that range from mild rashes and hives to life-threatening anaphylactic shock. Peanut allergy is the leading cause of lethal or near-lethal allergic reactions, and it is one of the most common food allergies in Canada. Data from a recent nation-wide survey indicates that approximately 2% of Canadian children, and 1% of Canadians overall, are allergic to peanuts. Not only can the condition be severe, it is usually lifelong; only 20% of those who develop the allergy early in life will eventually outgrow it.

A Suspicious Gene

AllerGen investigators Dr. Andrew Sandford, a professor and researcher at the University of British Columbia's James Hogg Research Centre, and Dr. Catherine Laprise, an expert in the genetics of asthma and allergies at the Université du Québec à Chicoutimi, recently led a study that aimed to shed more light on the genetic contribution to peanut allergy. Their findings provided important clues to understanding the origins and development of this disorder.

Of the more than 20,000 genes found in human DNA, Drs Sandford and Laprise chose to explore one in particular, the HLA-DQB1 gene (or 'HLA').

They chose this particular gene for two reasons: First, from previous research, they knew that peanut allergy, like other allergic diseases, is essentially an immune system response gone awry. Meant to protect the body against hostile invaders like viruses and bacteria, the immune system recognizes peanuts not as a food, but as a threat. The body reacts by unleashing a series of defensive actions, triggering inflammatory responses that may cause swelling, skin irritations, cramps, nausea or vomiting, wheezing and difficulty breathing.

The HLA gene is part of the immune system responsible for identifying an incoming substance as 'friend' or 'foe.' According to Dr. Sandford, there are potentially thousands of genetic variations when it comes to the HLA gene. "HLA is one of the most variable genes," he explains. "It needs to be, for the immune system to be able to deal with many different things. We surmised that there may be variations of this gene that contribute to a dysfunctional immune system response to peanut."

Second, other studies have shown an association between the HLA gene and asthma. "The association was very convincing," says Dr. Sandford. "Asthma can be an allergic disease, and there certainly are similarities between different types of allergic disease. So, looking for an association between the HLA gene and peanut allergy seemed like a good idea."

Although three previous studies have delved into a possible association between the HLA gene and peanut allergy, they produced contradictory results. Drs Sandford and Laprise were not deterred by this. "The results from previous studies were probably contradictory because the sample sizes — less than 100 samples — were small. It's hard to get reliable data when the sample size is small. Since we planned to have a much larger sample size, we thought that we could get a more definitive answer about the involvement of the HLA gene in peanut allergy than previous studies had provided," says Dr. Sandford.

In fact, this AllerGen-funded research project was the largest genetic study of HLA and peanut allergy in children ever conducted, involving more than triple the number of peanut allergy cases previously analyzed.

Exploring HLA-DQB1

Drs Laprise and Sandford enlisted the collaboration of AllerGen researchers Drs Ann Clarke and Yuka Asai (McGill University Health Centre), and Dr. Peter Hull (University of Saskatchewan) to provide DNA extracted from the saliva samples of 590 children with peanut allergy. For comparative purposes, DNA extracted from blood samples from 332 children without peanut allergy or any other allergic disease were obtained from two other genetic cohorts — the Canadian Asthma Primary Prevention Study (CAPPS) and the Study of Asthma, Genes and the Environment (SAGE), both led by AllerGen investigators.

Dr. Laprise's laboratory evaluated DNA from the saliva and blood to determine which genetic variants of HLA were present. "The high variability in this gene in terms of motif makes it highly complex to evaluate relative to other genes. In fact, it

The HLA gene is part of the immune system responsible for identifying an incoming substance as 'friend' or 'foe.' According to Dr. Sandford, there are potentially thousands of genetic variations when it comes to the HLA gene. "HLA is one of the most variable genes," he explains. "It needs to be, for the immune system to be able to deal with many different things. We surmised that there may be variations of this gene that contribute to a dysfunctional immune system response to peanut."

requires unusual technical and analytic tools to discriminate between the numerous possible variation patterns," says Dr. Laprise. Then, the researchers compared the frequency of HLA variants in the DNA of allergic children to that of children with no allergy. "We found highly significant differences between the two groups," says Dr. Sandford.

One Step Closer

The researchers discovered that two specific variants of the HLA-DQB1 gene were responsible for an increased risk of peanut allergy, while two other variants actually protected against the condition. "These results suggest that the HLA-DQB1 gene is involved in the development of peanut allergy and that variants in this gene are responsible, in part, for some people being susceptible to this disorder," says Dr. Sandford.

However, while HLA variation may indicate a genetic inclination to peanut allergy, it is not a definitive predictor. "Having a particular variant of HLA does not mean that you will definitely develop peanut allergy," explains Dr. Sandford. "It's not that simple. But it does increase your risk of becoming allergic to peanuts. There are clearly other factors involved — probably other genetic factors along with environmental ones."

Even so, by identifying certain variants of the HLA-DQB1 gene as a genetic risk factor, this AllerGen study has helped the research community edge closer to solving the mysteries of peanut allergy. Understanding how genes predispose people to this complex allergy will help researchers find new ways to fight the condition. Since people with peanut allergy often also have asthma and other immune-related disorders, these genetic findings may benefit other patient groups as well.

"This was only the second study to definitively show that there is a genetic factor that predisposes a person to developing peanut allergy," states Dr. Sandford. "Given our prior knowledge of the HLA gene, our results were not entirely surprising, but it is a very important step in the quest to solve the puzzle that is peanut allergy."

Experts estimate that 3.5 million tonnes of phthalates are produced globally each year for use in the manufacture of a vast array of everyday household products.

A New Approach to Measuring Indoor Exposure to Phthalates

Avoiding contact with phthalates ("THAL-ates") is nearly impossible. Step inside your home and you are surrounded by them — human-made chemicals designed to improve flexibility, transparency, durability or smell in a broad array of common consumer goods. They help cosmetics spread, vinyl stay flexible and bottled shampoo retain its fruity scent. They are used so widely and so often that they are often not named in ingredient listings, making their presence difficult to detect.

Dr. Miriam Diamond, Professor, University of Toronto

Phthalates are found in shower curtains, perfumes, bubble bath, bug sprays, cosmetics, clothing, food containers, wrapping, and, surprisingly, in trace concentrations in people who have been tested for the chemical.

A problem, scientists believe, because phthalates may also be making us sick.

Our Plastic World

Experts estimate that 3.5 million tonnes of phthalates are produced globally each year for use in the manufacture of a vast array of everyday household products.

However, because phthalates are not chemically bound to the products in which they are found, they leach into the surrounding environment and are easily absorbed by our bodies. Evidence suggests that, once absorbed, phthalates contribute to the development of asthma and allergies in children and block the production of testosterone (a male hormone that is key to growth, development and overall health) in the fetus.

Dr. Miriam Diamond, a professor at the University of Toronto, studies environmental contaminants and is troubled by the level of phthalates to which we are regularly exposed. "Since Canadians spend up to 90% of their time indoors, we have ample opportunity for exposure through this environment," explains Dr. Diamond. "Our research focuses on investigating indoor concentrations of phthalates, estimating emissions by means of mathematical modeling, and understanding factors that control these concentrations. This knowledge will be used to devise means of reducing our exposure to these compounds."

With allergic disease and asthma on the rise in Canada and globally, Dr. Diamond is hoping to develop a low-cost, low-tech sampling method to measure indoor phthalate levels — something that would be welcomed by phthalate researchers worldwide.

Phthalate exposure is just one factor under investigation in the AllerGen-funded Canadian Healthy Infant Longitudinal Development (CHILD) Study, which has been tracking a national cohort of children since before they were born to shed light on how allergic disease and asthma develop in early childhood.

The CHILD Study recruited 3,600 pregnant women and collected a wealth of data about the pre-birth environment, including information on pets, dust and phthalate levels, nutrition, and the mothers' physical and mental health status, in an effort to learn as much as possible about genetic and environmental influences on the fetus. After birth, the children are clinically assessed at three months and at one, three and five years of age. CHILD Study researchers are tracking the impact of individual genetic and environmental factors on the development of asthma and allergies in the children as they grow.

Measuring phthalate exposure for the more than 3,000 children involved in the CHILD Study is a complex, time

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"There is still so much that we don't know about the health effects of phthalates," says Dr. Diamond. "Should exposure in the womb be our primary concern, or should it be exposure after birth? What are the long-term effects?"

consuming and expensive proposition. Phthalate levels are usually detected with a urine sample — difficult to extract from the diaper of a three-month-old, and pricey at a laboratory analysis cost of $300 per sample.

So, with the CHILD Study and other investigations in mind, and with AllerGen NCE's support and funding, Dr. Diamond set out to develop an easy-to-use, accurate and inexpensive method for estimating indoor exposure to phthalates.

Trapping Phthalates with Wipes and Foam

As part of the investigation, Dr. Diamond collaborated with other experts in the AllerGen Network, including Dr. Tim Takaro (Simon Fraser University), Sri R. Chaudhuri, Amandeep Sanai and Dr. Emma Goosey (University of Toronto), Dr. Jeffrey Brook (Environment Canada) and Dr. Amanda Wheeler and Angelika Zidek (Health Canada). This interdisciplinary team provided expertise in three critical areas: air quality measurement and analysis; human exposure measurement and analysis; and analytical chemistry.

Dr. Diamond and her team selected five Toronto homes to test for indoor phthalates and other indoor contaminants over a four-day period. This was done twice using two unique sampling methods. The first method employed a laboratory version of tissues called "Chemwipes" to obtain a sample of surface grime. "Any chemicals released into the air eventually settle and contribute to household surface grime," says Dr. Diamond. Initially, the team planned to use the Chemwipe on the surface of coffee tables; however, they realized that if phthalates had been used to manufacture the coffee table, the results would be distorted. Since glass does not contain phthalates, the researchers decided to obtain grime samples from the surface of the homes' windows.

The research team also used Chemwipes to sample the hands and forehead of each adult in the home. Since we touch

phthalate-containing products all day long, they wondered if traces of the chemical would be found on subjects' hands. With the forehead samples, the team wanted to determine if airborne phthalates accumulate on skin as they do on other household surfaces. "Other than the human mouth and eyes, phthalates penetrate the skin slowly," explains Dr. Diamond, "With a constant supply of phthalates entering the indoor environment from products, there's a constant supply to your skin."

The second sampling method used a polyurethane foam — exactly the same material that makes couch, chair and car seats squishy. Left in a room, the foam acts as a passive air sampler. "Polyurethane foam is amazing for absorbing chemicals," states Dr. Diamond. "It is unbelievable. It's also cheap and easy to use."

Using the Chemwipes and the foam, the team obtained measurements of phthalate concentrations in surface films, air and dust in two to three rooms per home. They also took into account other characteristics of the homes' indoor environment, such as how quickly indoor air exchanges with outdoor air, temperature, particulate matter concentration and dwelling dimensions. Adults living in each home completed a questionnaire and provided a daily urine sample and skin wipes to be used as biological measures of phthalates and for comparison with the levels found on window wipes and in foam.

Surprising Preliminary Results

The team's research plan called for the collected samples to be analyzed in an Environment Canada laboratory. However, severe federal budget cuts eliminated this option. "The funding cut to Environment Canada was deep and dramatic and it greatly set back our research project," Dr. Diamond recalls. Eventually, a new partnership was formed with Dr. Hong Qi, a scientist at the Harbin Institute of Technology in China, who taught Dr. Diamond's students an analytical method by which to process their samples.

Preliminary results suggest that the window wipes provide a good indication of indoor phthalate concentrations, at least for one particular type of phthalate. "We were surprised to discover that," says Dr. Diamond. Phthalates were also measured in all skin wipe samples. Some forehead samples registered particularly high levels, which may have been transferred from hair products, according to Dr. Diamond.

Dr. Diamond believes that, given the myriad of phthalates in use today, multiple sampling methods may ultimately be needed to measure their presence accurately. "One of the reasons we don't have all the answers yet is that it is so incredibly challenging to measure phthalates," says Dr. Diamond. "They are everywhere. In fact, even the equipment we use to extract samples is full of one type of phthalate."

The team also found that all rooms in the five homes tested contained similar levels of phthalates, which was surprising. One exception was a home in which renovations were underway; that home had much higher levels of all phthalates measured. Dr. Diamond also suggests that phthalate levels would likely vary across households of differing socio-economic standing. "It's easy to imagine that a trailer home with a vinyl interior will have much higher levels of phthalates than a well-to-do home with upscale furnishings," she says.

'Off the Wall' Research

Dr. Diamond is grateful for AllerGen's support of this project. "This was an unconventional project. Often, research funding is funneled towards the 'conventional,' which constrains our ability to discover," she notes. "Thankfully, AllerGen was willing to fund this 'off the wall' research, like the usefulness of window wipes," she says, and then adds, "But please excuse the pun."

While Dr. Diamond's efforts to develop an effective and cost-efficient sampling method will benefit large-scale epidemiological studies like the CHILD Study, her research also has broader economic, educational and policy implications. "There is still so much that we don't know about the health effects of phthalates," says Dr. Diamond. "Should exposure in the womb be our primary concern, or should it be exposure after birth? What are the long-term effects?"

Hopefully, the answers to these questions, obtained through studies like this one, will influence public policy and pressure industry to develop safer alternatives to phthalates and other toxic chemicals. "There is a lot of concern, particularly from parents, about chemicals in the home," Dr. Diamond remarks. "Our goal is to help people understand and put into context the scary information that they hear and read, in order to enable and empower them to know what to do about their chemical exposure. I see that as being really important."

"The ultimate question is: Could having rhinitis predispose you to developing asthma and, if so, how can we prevent its development?"

United Airways: From Nose to Lungs

"United Airways" may sound like the name of an airline, but to the world's top allergy researchers like Dr. Louis-Philippe Boulet, it means something completely different. United airways is a relatively new way of thinking about the relationship between the nose (the upper airway) and the lungs (the lower airway). Despite the obvious physical connection between the two — air enters through the nose and passes through the airway into the lungs — conditions afflicting the nose and lungs have always been evaluated and treated as separate disorders.

Dr. Louis-Philippe Boulet, Professor of Medicine, Université Laval, Respirologist, Institut universitaire de cardiologie et de pneumologie de Québec

Over the past decade, research has emerged showing that the upper and lower airways are united in ways that go beyond simply providing a transportation system for inhaled air. The concept of united airway disease (UAD) recognizes that upper airway problems, such as allergic rhinitis (an inflammation of the nose's mucous membrane), and lower airway conditions, such as asthma, are intimately connected. In fact, some conditions that affect both airways may best be understood as a single disease. The concept is simple: inflammation in the nose affects inflammation in the lung and, in some cases, nasal inflammation may contribute to the development of asthma.

Dr. Boulet, a physician from Université Laval and an AllerGen Principal Investigator, is an expert in understanding and treating asthma, allergic rhinitis and nasal polyps — those small, usually harmless growths that protrude from the mucous membrane of the nose. Responding to the groundswell of interest in UAD, Dr. Boulet set out to investigate how allergic rhinitis and nasal polyps influence inflammation in the lower airways.

With support and funding from AllerGen NCE, and in collaboration with AllerGen colleagues Drs Paul Keith and Judah Denburg at McMaster University, Dr. Boulet and his team, including researcher Marie-Ève Boulay and graduate student Marie-Claire Rousseau, conducted a series of studies on the influence of nasal allergen challenges on lower airways.

"Since we know that a significant number of people develop rhinitis before becoming asthmatic, we wanted to see if rhinitis contributes to the very development of asthma," says Dr. Boulet. "The ultimate question is: Could having rhinitis predispose you to developing asthma and, if so, how can we prevent its development?"

Pass the Tissues Please

Allergic rhinitis, with its eye-rubbing and nose-blowing symptoms, can be triggered by viruses, bacteria or a variety of allergens. Rhinitis is common: over five million Canadians, or more than one in six, suffer from the condition. Many people with rhinitis are asthmatics, and over 80% of people who suffer from asthma also have allergic rhinitis.

For their study, Dr. Boulet and his team recruited 41 people diagnosed with allergic rhinitis, approximately half of whom also suffered from asthma. Over four consecutive days, the subjects were exposed to low doses of an allergen — cat hair or dust mites (the microscopic insects found in house dust) —to trigger an allergic reaction.

For their study, Dr. Boulet and his team recruited 41 people diagnosed with allergic rhinitis, approximately half of whom also suffered from asthma. Over four consecutive days, the subjects were exposed to low doses of an allergen — cat hair or dust mites (the microscopic insects found in house dust) — to trigger an allergic reaction.

Subjects received one squirt of the allergen in each nostril and symptoms were recorded 10 minutes later. Upper and lower airway inflammation was evaluated on the first and fourth days of exposure. Researchers watched closely to see if the study participants who developed rhinitis would go on to develop lower airway inflammation. In fact, 15% of the subjects (one in six) did and it is possible, according to Dr. Boulet, that using an increased dose of allergen would have pushed this number even higher.

Among the subjects with asthma, the effect of triggering allergic rhinitis either induced a new inflammatory response in the lower airway or increased a pre-existing one. "This supports the idea that there is a close link between the upper and lower airways," states Dr. Boulet, "and also underlines the possibility that rhinitis could eventually promote the development of asthma, although long-term studies are needed to confirm this."

Dr. Boulet and his team also found that people who have rhinitis and nasal polyps display an 'amplified' lower airway inflammatory response. "People with nasal polyps have an amazingly intense lower airway response, even if they don't have asthma," says Dr. Boulet. Nasal polyps are not always visible to the untrained eye, but people who have them feel as though they have a permanently blocked nose. If further studies confirm Dr. Boulet's findings, polyps may be considered a risk factor for asthma.

Results that are Nothing to Sneeze at

"We achieved a great deal with a small amount of money," Dr. Boulet says of the team's achievements, which he believes extend far beyond the findings of the study.

To help with the recruitment of subjects for the study and to better quantify the disease, Dr. Boulet and his team designed a questionnaire to quickly identify patients with rhinitis. It was

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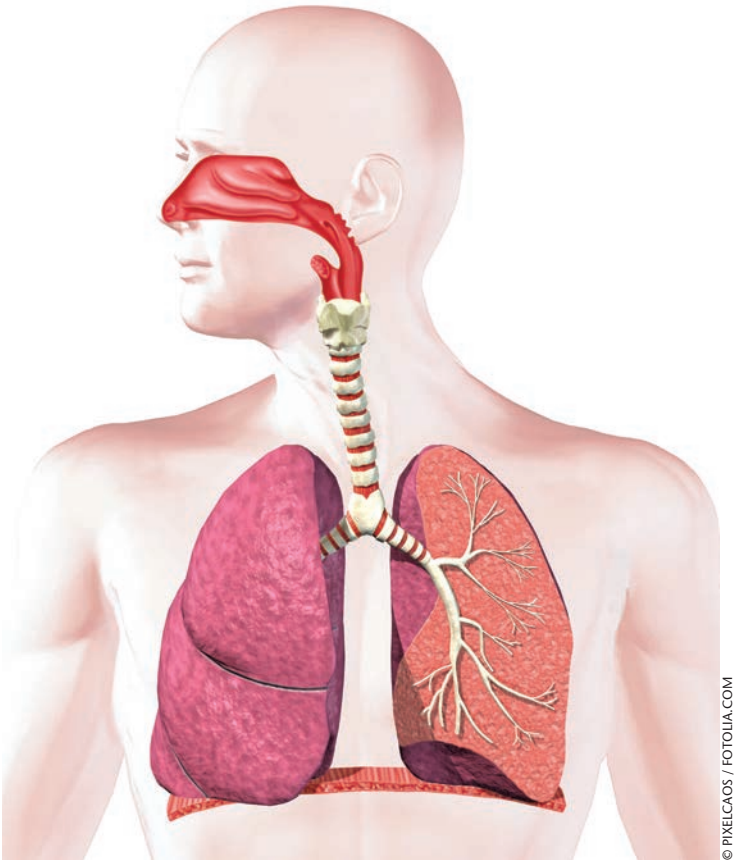

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modeled on an existing questionnaire used to quantify asthma control. Work continues to combine the two questionnaires into one — and simplify it for easy use by physicians — so that allergic patients can have an assessment done of both the upper and lower airways simultaneously. "This tool should improve the recognition and management of these conditions," states Dr. Boulet. "We will disseminate this tool as quickly and widely as possible after it is standardized."

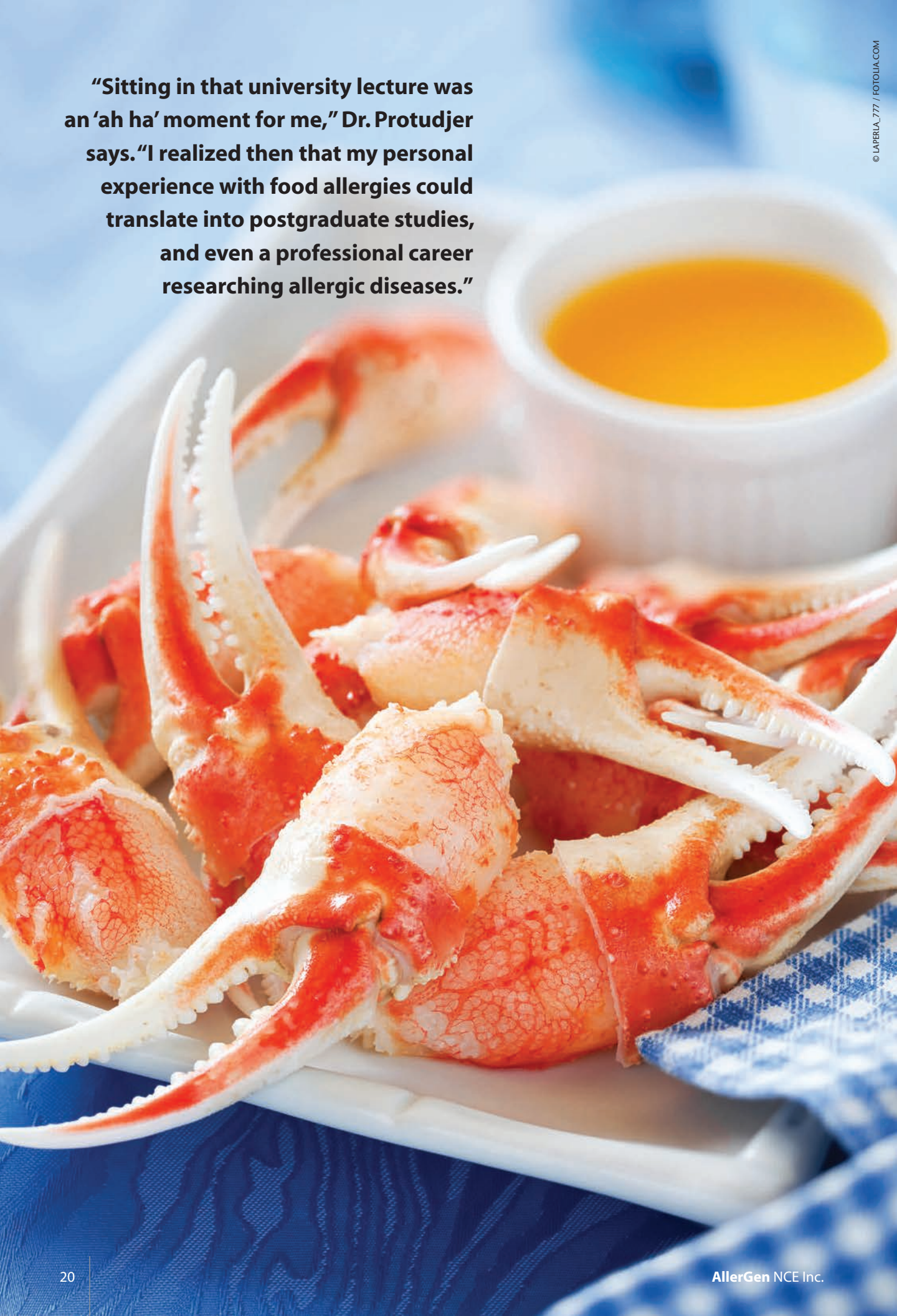
As well, the researchers are exploring new, non-invasive techniques for measuring lung inflammation. For years, researchers have relied upon invasive bronchoscopies (which use inserted scopes to visualize a patient's airway) and bronchial biopsies to diagnose airway disease. "It's not easy to do invasive techniques repeatedly," Dr. Boulet notes. Non-invasive techniques of inflammometry (the measurement of inflammation), developed by researchers such as the late Dr. Frederick Hargreave, now offer a more practical alternative.

Dr. Hargreave's group at McMaster University was instrumental in standardizing induced sputum analysis as a non-invasive method for measuring airway inflammation. This technique requires subjects to inhale a salt solution, causing them to cough up phlegm (or sputum), which is collected and analyzed. "By refining this test, and possibly identifying other means of non-invasive measurements of airway inflammation," comments Dr. Boulet, "we will be able to make such measures more easily available in the future."

Finally, this AllerGen-supported project spawned collaborations and exchanges between researchers and AllerGen trainees from McMaster and Laval Universities. Fostered by AllerGen NCE, this team of researchers continues to refine the methodology for nasal provocation and to further explore the relationship between rhinitis and asthma.

Inspired by their results, Dr. Boulet hopes that he and his colleagues will eventually discover biomarkers that indicate who is at high risk of developing asthma. "Being able to prevent asthma would really be something," says Dr. Boulet. "We dream about that."

"Sitting in that university lecture was an 'ah ha' moment for me," Dr. Protudjer says. "I realized then that my personal experience with food allergies could translate into postgraduate studies, and even a professional career researching allergic diseases."



AllerGen: An Incubator for Young Researchers

Jennifer Protudjer remembers the moment clearly. Her university professor was describing anaphylaxis — a severe, potentially life-threatening allergic reaction — and suddenly she knew she wanted a career in research studying allergies and immune diseases.

Then a third-year University of Manitoba undergraduate, Protudjer had lived with allergies to fish and shellfish all her life and had experienced first-hand the challenge of managing a condition that many people don't understand or take seriously. As a child, the Winnipeg-born Protudjer became adept at avoiding her trigger foods and teaching others about her allergies and the importance of proper management.

Dr. Jennifer Protudjer, PhD, Postdoctoral Fellow, Karolinska Institute

"Sitting in that university lecture was an 'ah ha' moment for me," Dr. Protudjer says. "I realized then that my personal experience with food allergies could translate into postgraduate studies, and even a professional career researching allergic diseases."

With encouragement from an academic mentor and support from AllerGen NCE, Protudjer followed precisely this path. Now a Postdoctoral Fellow at the Karolinska Institute in Stockholm, Sweden, one of the world's leading medical universities, Dr. Protudjer studies the intersection of asthma and puberty, and whether or not the use of childhood asthma medication influences pubertal growth and development.

A Launching Pad

In her first year of graduate school, Dr. Protudjer's supervisor at the University of Manitoba, Dr. Allan Becker, introduced her to the AllerGen Network. An internationally recognized expert on the origins of allergy and asthma in early life, Dr. Becker was also an AllerGen Principal Investigator and the leader of the Study of Allergy, Genes and the Environment (SAGE) project — a nested case-control study of 723 Manitoba children born in 1995. Using questionnaire and clinical data, SAGE examined the relationship of early-life exposures to the development of asthma.

For her Master's thesis, Dr. Protudjer interviewed SAGE participants and their families to better understand their perceptions of asthma. One of her key findings was that adolescents (11-13 years) with asthma tend to 'normalize' their condition much the same way that young people bound to a wheelchair or living with cystic fibrosis normalize theirs. By taking asthma medications and modifying how they participate in physical activity, youth with asthma are able to pursue what they perceive to be a 'normal' way of life for a person of their age.

"Working on the SAGE project gave me important clinical experience working directly with children and families living with food allergies and asthma, and let me benefit from a wealth of multidisciplinary collaborations with researchers in pediatric allergy, population health, immunology, and genetic and environmental epidemiology," Dr. Protudjer says.

"Working on the SAGE project gave me important clinical experience working directly with children and families living with food allergies and asthma, and let me benefit from a wealth of multidisciplinary collaborations with researchers in pediatric allergy, population health, immunology, and genetic and environmental epidemiology," Dr. Protudjer says. Funding provided by AllerGen, the Canadian Institutes of Health Research (CIHR) and other organizations freed the young graduate student from the need to find additional employment, allowing her instead to devote her time to research and coursework.

Her findings suggest that children — particularly those who are overweight —who spend at least one hour per week of 'screen time' (viewing television, playing video games, surfing the net, *etc.*) at eight to 10 years of age are much more likely to develop asthma by the time they are 12 to 13 years old.

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Throughout her PhD studies, Dr. Protudjer's research continued to explore how teens deal with asthma. She designed a unique 'mixed methods' study that combined analyses of body weight, quality of diet, puberty, physical activity and sedentary behaviour (quantitative data), together with personal interviews and focus group discussions with teens and their parents (qualitative data), to produce a more complete picture than either method alone could reveal about how youth cope with their condition. Dr. Protudjer found that physical activity levels had no influence on the youths' experience of asthma. However, her findings also suggest that children — particularly those who are overweight — who spend at least one hour per week of 'screen time' (viewing television, playing video games, surfing the net, *etc.*) at eight to 10 years of age are much more likely to develop asthma by the time they are 12 to 13 years old.

"Rising Star"

Dr. Protudjer views her participation in AllerGen as a critical turning point in her career development. "Dr. Becker encouraged me to take advantage of the extensive training, networking and capacity building opportunities that the AllerGen Network offers to students and trainees. That was one of the best pieces of advice I have ever received," she says.

Dr. Protudjer joined the AllerGen Students and New Professionals Network (ASNPN) — a national network of student trainees and junior professionals involved in allergic disease research. Founded in 2005, the ASNPN offers a suite of capacity building programs and events designed to support, educate and mentor trainees. Dr. Protudjer flourished within the Network, taking advantage of the full range of AllerGen trainee opportunities, including the travel awards program (which facilitates participation in national and international conferences), poster competitions highlighting student research, lab exchanges to acquire new research skills, and networking and skills training at annual trainee symposia.

"As an AllerGen trainee, I gained a diverse skill set and access to countless opportunities that otherwise would not have been available to me," Dr. Protudjer says. In particular, two unique leadership opportunities laid the groundwork for her eventual jump to an international research position in Sweden.

Jennifer Protudjer is recognized for her leadership as ASNPN President by Mr. Graham Scott, Chair, AllerGen Board of Directors, 2012

In 2009, Dr. Protudjer became President of the ASNPN, gaining valuable leadership experience, and affording her the opportunity to serve on AllerGen's Board of Directors, among other senior Network advisory committees. Dr. Protudjer's term as ASNPN President culminated with her involvement in AllerGen's NCE funding renewal application, as part of which she addressed a panel of international research experts reviewing the Network for the NCE Directorate on the value and strength of the Network's capacity building programs. Following her presentation, the Chair of the NCE Renewal Committee commented that Dr. Protudjer was "clearly a rising star among the next generation of researchers."

A year later, Dr. Protudjer participated in AllerGen's International Partnership Initiative, taking part in a six-week exchange to the Karolinska Institute. This helped Dr. Protudjer become a globally-engaged scientist capable of performing in an international research environment, and led to her post-doctoral fellowship at the Karolinska Institute (funded by the European Respiratory Society and CIHR), where she worked with Dr. Catarina Almqvist Malmros.

Globe-spanning research

While much of Dr. Protudjer's work focuses on asthma in children and teens, she remains passionate about helping people of all ages manage their food allergies. With an international team of Swedish and Canadian researchers, Dr. Protudjer is helping to develop a novel food allergy education program for young people. As a member of the FoodHE team at the Karolinska Institute (part of the larger EuroPrevall Study), she will continue to explore and publish on the connections between food allergy, quality of life and socio-economic cost.

With many new and innovative projects on the horizon, Dr. Protudjer believes that her relationship with AllerGen will help her to continue to address pressing issues in the diagnosis, treatment and management of asthma, allergies and related immune diseases. "My professional goals would very likely not have been realized without the mentorship of Dr. Allan Becker and the involvement of AllerGen," Dr. Protudjer reflects. "I am so grateful that I belong to the AllerGen community of researchers, who are recognized around the world. This unique opportunity is of great benefit to me now and I expect it will continue to be beneficial to my work for many years to come."

Success Stories

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