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UNIVERSITY OF OTTAWA
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INSTITUT DE CARDIOLOGIE
DE L'UNIVERSITÉ D'OTTAWA

THE BEAT™

A COMPENDIUM OF INFORMATION ABOUT THE UNIVERSITY OF OTTAWA HEART INSTITUTE

HIGHLIGHTS

The identification of 9p21 is regarded as one of the most important discoveries in the genetics of cardiovascular disease.

(from The Genetics of Coronary Artery Disease and Its Metabolic Risk Factors, page 7)

Dr. Gollob is the leader of a group of Canadian cardiologists who have developed the first guidelines in the world for genetic testing in the clinical evaluation of inherited arrhythmias associated with sudden cardiac death, on behalf of the Canadian Cardiovascular Society and the Canadian Heart Rhythm Society.

(from Translating Genetics into Better Patient Care, page 7)

In fact, the Ruddy Centre is part of what convinced this rising star to choose the Heart Institute after completing two post-doctoral fellowships at Harvard.

(from Uncovering the Genetic Mysteries of Heart Development, page 8)

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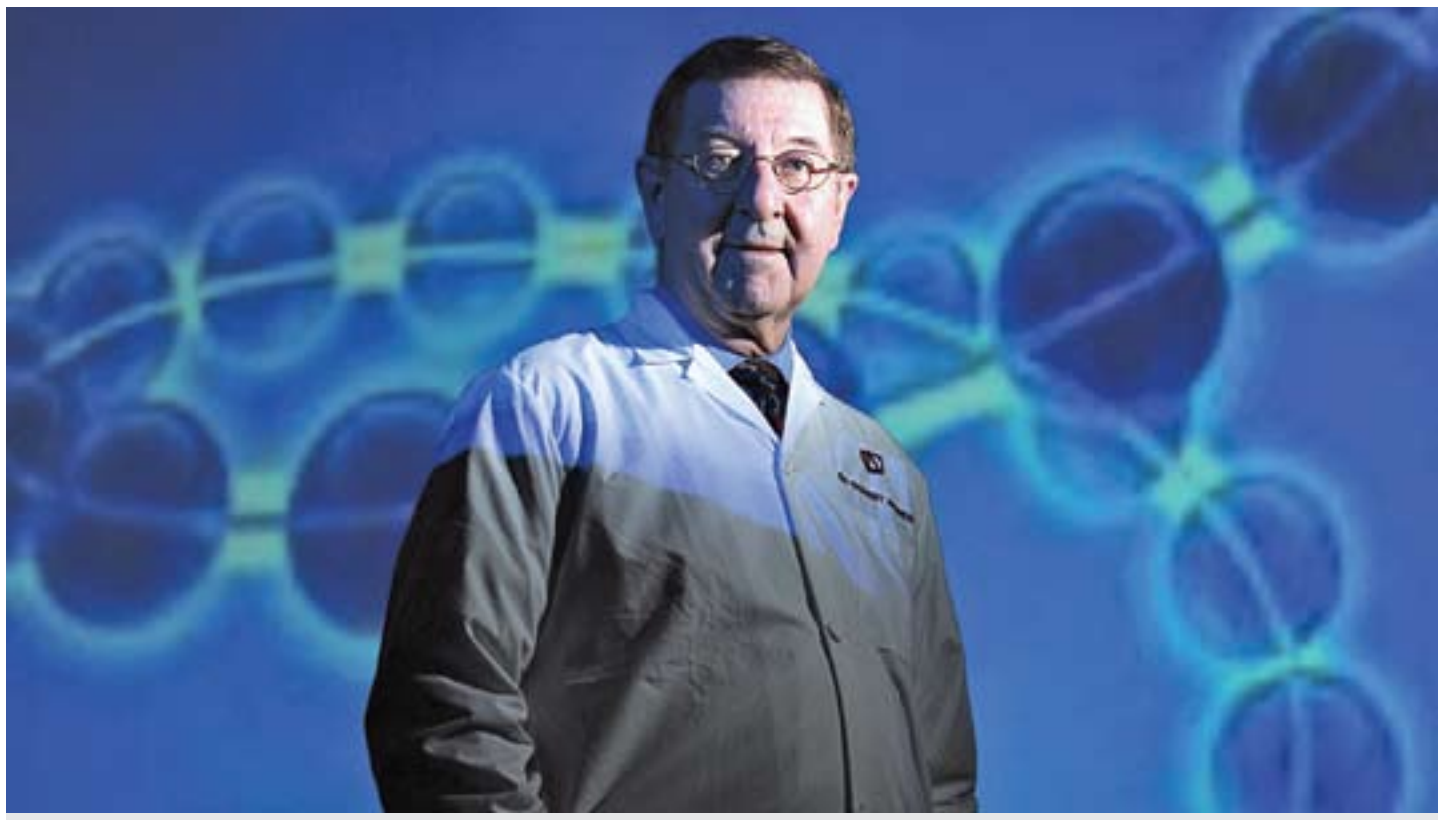


Dr. Robert Roberts, President and CEO of the University of Ottawa Heart Institute, is a renowned cardiologist and a pioneer in the genetics of heart disease. His founding of the Ruddy Canadian Cardiovascular Genetics Centre in 2005 has led to a continuing series of important genetic discoveries.

Dr. Robert Roberts: Towards Personalized Prevention and Treatment

Dr. Robert Roberts joined the University of Ottawa Heart Institute as President and Chief Executive Officer in 2004, after serving as Chief of Cardiology at Baylor College of Medicine. He has also served as Director of the Ruddy Canadian Cardiovascular Genetics Centre since its founding in 2005. An expert in cardiovascular genetics, Dr. Roberts previously participated in the discovery of several rare inherited **genetic mutations** that cause cardiovascular disease. He now leads the Ruddy Centre in the search for more common **genetic variations** that increase the risk of heart disease in the general population.

While many institutions have chosen to outsource the data analysis for large-scale genetic studies, the Heart Institute chose to build its own facility—the Ruddy Centre—to give its researchers the capability to perform large **genome-wide association studies (GWAS)** in-house.

"The problem with outsourcing is that it doesn't develop your own researchers' skills, and the interpretation and analysis of the data is not being done where the data is being generated," said Dr. Roberts. "We had the expertise needed to develop a large, high-throughput genetics centre, and we felt that if we wanted to be at the cutting edge of research, we needed to be directly contributing. Part of my mandate when I came here in 2004 was to help the Heart Institute really compete, research-wise, at the national and international level."

"For research, and for the Heart Institute in general, I think the Centre has been a major windfall, and it's been one of the best integrators for bringing people together in our research program—bench scientists, cardiologists, imaging experts, statisticians—all of these people are working and publishing together, and results have materialized much faster than we ever expected," he added.

With the current breakneck pace of genetic discovery coming out of international collaborative GWAS, many involving Ruddy Centre participation, Dr. Roberts thinks it's not far fetched to believe that the majority of genetic variations associated with the risk of heart disease will be **mapped** within several years.

The next and far more complicated step, he explained, is to understand how each genetic variation actually exerts its effect on heart health—how it either predisposes an individual to or protects him or her

(continued on page 4)

Editor's Note

Welcome to a unique edition of *The Beat!* We have assembled a special issue on genetics at the Heart Institute to celebrate the fifth anniversary of our world renowned Ruddy Canadian Cardiovascular Genetics Centre. The Ruddy Centre is only one of a handful of genetics centres worldwide that focus exclusively on the genetics of cardiovascular disease. In just a few short years, the Ruddy Centre has more than made its mark on the field.

Understanding genes, their function and their influence on heart disease is perhaps one of the most significant research endeavours being undertaken today. Cardiovascular genetics is also a relatively young science and its promise is profound. These pages detail the genetics effort currently underway at the Heart Institute and document its promise for us all.

Genetics involves a great deal of terminology specific to the field. To aid those unfamiliar with the language of genetics, we have **highlighted key terms in red** throughout the issue. These are defined in the feature "The Language of the Genome" on page 2.

The Language of the Genome

The **genome** is the complete genetic code for a living organism. This code is "written" in the language of **DNA**. The alphabet of this language consists of four different types of molecules called nucleotides, each represented by a letter. These four letters—A, C, G, T—can be combined in nearly endless combinations to spell out the genetic instructions for the organism's development and functioning. Nucleotides in DNA exist as **base pairs**—two nucleotides joined together that form the ladder rungs of DNA's molecular structure.

The **human genome** has an estimated 20,000 to 25,000 genes stored on 23 chromosome pairs. **Chromosomes** are the structures within cells that hold DNA together and help control gene expression. A **gene** is a section of DNA that directs the production of a protein or RNA in the body. Genes average about 20,000 base pairs that direct the production of a protein or RNA in the body. Everyone must have two copies of every gene. One from each parent.

Gene expression is the process through which the genetic instructions in DNA are used to produce working molecules within the body—proteins or functional RNAs. **Proteins** control how the body works. **RNAs** usually serve to carry the instructions for making proteins. **Regulatory RNAs** control how and when proteins are produced (i.e., expressed).

A person's **genotype** is the DNA blueprint for how he or she will look and function, and that person's **phenotype** is the physical form and function resulting from that genotype. Except for identical twins, no two people have the same genotype, due to both genetic variations and genetic mutations.

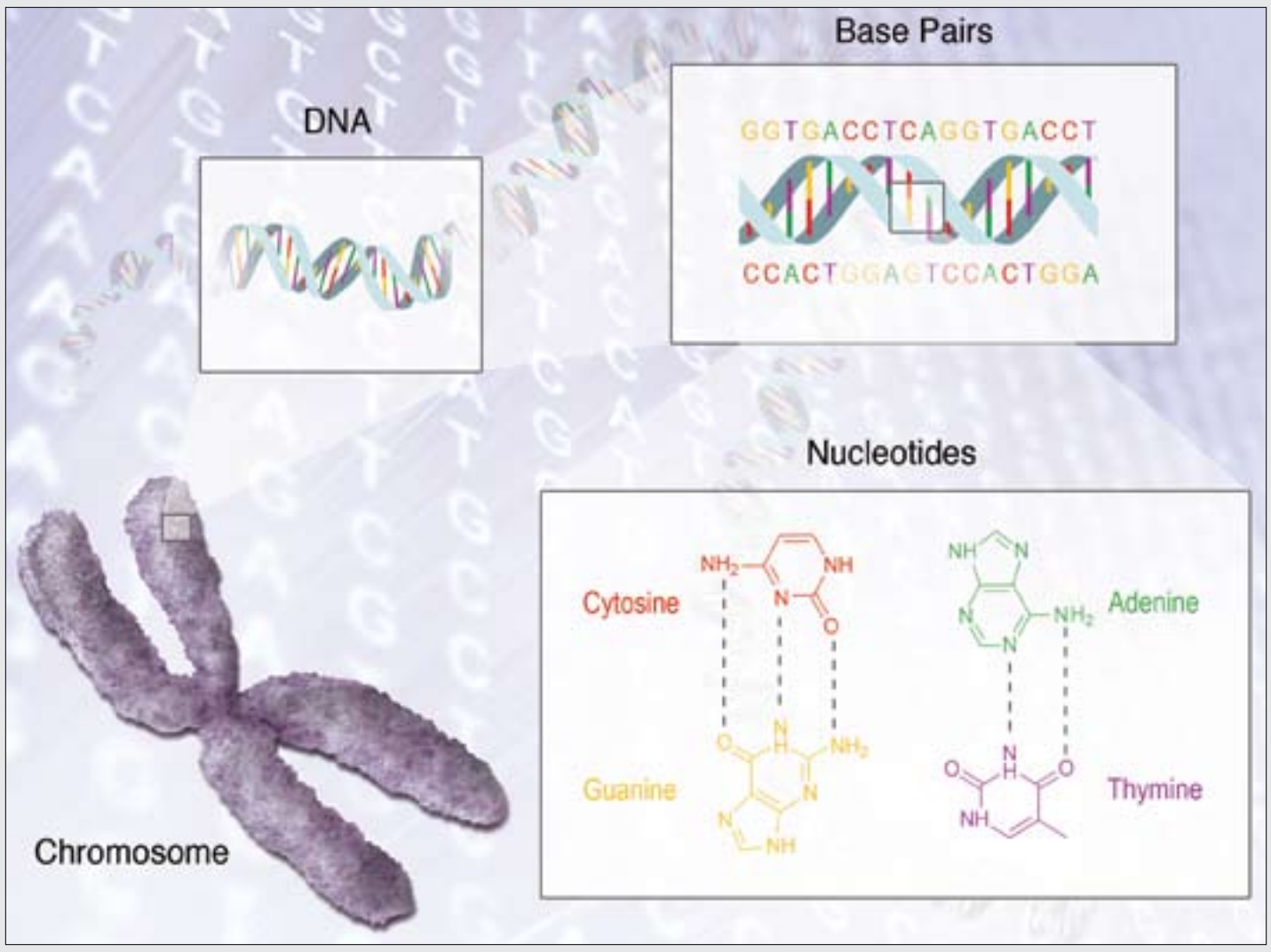
Each gene circulates in the general population in several forms. **Genetic variation** is the presence of commonly occurring alternate codings of specific genes. These result in differing physical traits and predispositions between individuals. An **allele** is a particular alternate coding of a gene. Each allele differs usually by only one base pair. It is alleles that cause people to have different hair colour or a greater likelihood of heart disease. A **genetic mutation** is a random change in the genetic code of an individual that, if passed on to the children, may eventually be incorporated into the population as a genetic variation.

Because both genetic variations and mutations influence a person's risk of disease, researchers in all fields of medicine are interested in finding genes associated with disease and understanding how they influence health.

Gene mapping is the process of searching for the physical location of a gene within the chromosomes. This process can lead to the discovery of a **genetic marker**: a gene or other DNA sequence known to be associated with a particular genetic trait and that has been mapped to particular location on a chromosome.

When looking for previously unknown genes that may influence health, researchers focus on **common variants**—alleles that occur in at least 5 per cent of the population being studied. These variants are hunted through a process called a **genome-wide association study (GWAS)**. A GWAS compares samples across large groups of people to look for associations between small gene variations, called **single-nucleotide polymorphisms (SNPs)**, and the risk of developing a particular disease. SNPs are variations in a single unit of the DNA code.

A GWAS relies on a type of technology called a **gene chip**, which can rapidly compare a DNA sample to up to a half-million pieces of DNA with known sequences. This and other processes that provide information on the contents of an individual's genetic code are known as **genotyping**. After identifying genetic variations associated with a disease through a GWAS, researchers may use **gene sequencing** to identify the order of nucleotides within that area of DNA to see if they match that of any known gene, or to begin the discovery process for a previously unknown gene.



We are who we are thanks to a combination of our genes and environmental influence. In the case of contracting heart disease, the consensus is that our genetic blueprint, or genotype, is responsible for about half of our risk. Understanding which genes are involved is essential to understanding and eradicating heart disease.

Building a Home for Cardiovascular Genetics

Since its launch in 2005, the Ruddy Canadian Cardiovascular Genetics Centre at the University of Ottawa Heart Institute has ushered in a new era in Canadian research into the genetic basis of heart disease. The Centre's focus on **genome-wide association studies (GWAS)** searching for common **genetic variants** associated with disease risk has led to a number of major findings in just a few short years.

By the late 1990s, the scientific community had identified the majority of single inherited **genetic mutations** that lead directly to cardiovascular disease. These include genes that cause various cardiomyopathies (weakening of the heart muscle), cardiac arrhythmias that can lead to sudden cardiac death, and familial high cholesterol. But these high-risk single mutations account for only a small minority of overall cases of heart disease.

"We were fully aware that common diseases, which is what we're most interested in, are due to more common genes, but until recently, the technology to look for these common genetic variations was not available," said Dr. Robert Roberts, Director of the Ruddy Centre and President and Chief Executive Officer of the Heart Institute.

Most of the remaining genetic variations contributing to heart disease risk will likely be **single-nucleotide polymorphisms (SNPs)**. SNPs are mutations in **DNA** which are found widely within and between populations throughout the world. SNPs are thought to largely account for the difference in susceptibility to various diseases observed among individuals.

SNPs contribute to only a low to moderate increase in disease risk—most falling under 10 per cent. Possessing one such variation alone will do little to alter an individual's risk of heart disease, but several together may make a large difference.

Until the past few years, genetic technology was unable to identify such small differences in the genetic code. However, recent advances have allowed the large-scale categorization of SNPs through GWAS (see "Five Years of Discovery", page 4) to become a reality.

In the early 2000s, the Heart Institute, like most other academic centres, had the desire but not the technology to delve into GWAS. Institute researchers had begun studies searching for SNPs associated with heart disease, but had to outsource the actual analysis of the samples they collected from patients. In 2005, the Institute chose to invest in the Ruddy Centre, to enable its researchers to perform large-scale genetic studies in-house and participate in international GWAS collaborations that began forming at that time.

Since its inception, the Centre has obtained approximately $20 million in direct funding, allowing for the purchase of high-throughput genetic equipment providing the capacity to quickly analyze and sequence massive amounts of genetic data (see Sidebar). This funding has also been critical for faculty training and participation in international research consortia. Today, the Ruddy Centre remains the only research centre in Canada dedicated to cardiovascular genetics and one of only a few in North America able to perform cardiovascular GWAS on-site.

(continued on page 3)

(Building a Home for Cardiovascular Genetics, continued)

From 2005 to 2010, staff has grown from one staff technician and one nurse coordinator to four of each. The Centre's researchers have fully trained two fellows and three graduate students, and two fellows are currently in residence. An additional 10 graduate students and 10 fellows from associated Heart Institute laboratories are directly involved in Centre research. The Ruddy Centre also brought on board the Institute's first full-time statistician.

To date, the Centre's researchers have published more than seventy papers, including the discovery of the first genetic risk gene for heart attacks (called 9p21). Additional findings are in press and in progress.

From its early days, the Ruddy Centre has collaborated in large, international GWAS examining the genetic risk of cardiovascular disease. International collaborative studies not only let the participating centres leverage their resources and more easily obtain the large patient populations required for GWAS research, but also enable results to be validated in diverse groups of people.

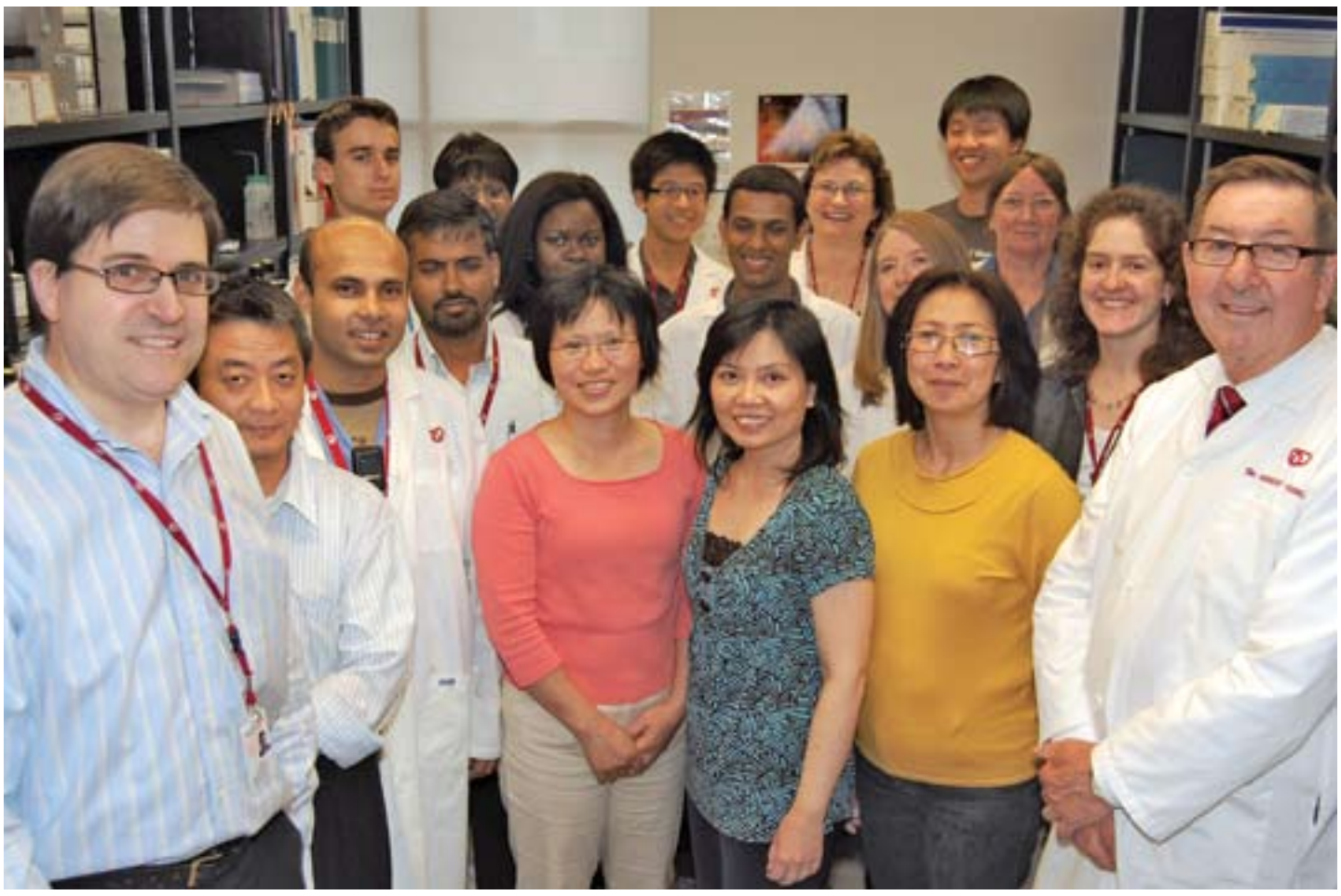
A problem with relying on any GWAS conducted in a single regional population, explained Alexandre Stewart, Principal Investigator of the Centre, is that there tends to be genetic regionalization; that is, people tend to share genetic traits with those who live closest to them. "So, to avoid discovering things that are unique to your regional population, you need to look farther out, to compare your findings with other, independent groups of people," said Dr. Stewart.

In order to achieve the large sample sizes in multiple population groups that are necessary for GWASs, international consortia have formed to share data sets. Through the Ruddy Centre, the Heart Institute has been an active, and in some cases founding, member of several of these groups, consisting of leading institutions around the world, including Harvard University, Massachusetts Institute of Technology, Stanford University, Oxford University, and the Wellcome Trust. Participation in these consortia is generally based on a track record of leading genetic research, the technical capacity to process large volumes of data and having a substantial library of genetic samples to contribute to the effort. Some examples include:

- Coronary ARtery DIsease Genome-wide Replication and Meta Analysis (CARDIoGRAM), which boasts a massive pool of 135,000 samples of individuals with or without coronary artery disease.
- Genetic Investigation of Anthropometric Traits (GIANT), whose goal is to identify all the genes involved in regulating height, weight and obesity
- HaemGen, which is looking to identify genes associated with blood parameters and their association to disease.

In addition to continuing the discovery process for additional genetic risk factors, an equally vital second phase of such studies is determining the function of the discovered genes.

"Mapping the location of genes is simply a means to an end. The end is finding those genes and determining what they do and then how we can counteract or, if they are protective, enhance their effect. The future, not just for this Institute, but the future of medicine for the next decade or 15 years is going to be very much tied up with determining the function of these newly discovered genes," said Dr. Roberts.



The staff of the Ruddy Canadian Cardiovascular Genetics Centre, led by Dr. Robert Roberts (right) and Alexandre Stewart (left), includes scientists, statisticians, technicians, graduate students and post-doctoral fellows.

Complicating this search for function is the fact that most of the human genome does not code for **proteins**. Originally thought to be so-called junk DNA that served no purpose, it is now known that the majority of this DNA is actually transcribed. Instead of proteins, though, it codes for **small regulatory RNAs** that affect how the rest of the genome is **expressed**.

To help unravel the complex pathways between gene variation and risk, Centre researchers are now exploring systems biology and other computational and mathematical approaches to understanding the complex molecular systems governing the progression of heart disease, in collaboration with colleagues at the University of Ottawa.

Researchers at the Centre are also building new animal models, including one of 9p21, that mimic the phenotype of human heart disease arising from newly discovered genetic variations, as a platform both for understanding the mechanisms by which the genes affect risk and for drug-discovery research. The Centre has 3,600 square feet of laboratory space dedicated to a future Genome Function Centre.

MORE INFORMATION ONLINE

Visit the Ruddy Centre Lab Page:

www.ottawaheart.ca/research_discovery/ruddy-canadian-cardiovascular-genetics-centre.htm

Growing High-throughput Capacity at the Ruddy Centre

Investment in state-of-the-art **gene sequencing** and **genotyping** technology enables the Ruddy Centre to process genetic samples at a pace unheard of even five years ago.

In 2005, the Centre could genotype 64 samples per week. Today, that number is more than 1,100 per week. The Centre is a designated Affymetrix Core laboratory, using the Affymetrix Gene Chip 6.0 platform.

The recently acquired Affymetrix Gene Titan platform and Axiom genotyping solution can process 96 samples at once and test 572,000 **genetic markers** per sample. The Ruddy Centre was the first laboratory in Canada with this technology.

The ability to process more than a thousand genetic samples per week allows Ruddy Centre investigators to genotype a group of patients large enough for a GWAS within just a few weeks. With several collaborators working at a similar pace in different populations, data can be validated and published extremely rapidly.

In 2005, the Centre's machines could sequence 1 million **base pairs** of DNA overnight. Today, that number is 50 million. The Centre possesses a Roche Genome Sequencer FLX, one of the most advanced sequencers available to date, as well as several moderate- and high-throughput sequencers that provide additional capacity.

The Centre's advanced sequencing technology will enable researchers to rapidly home in on and sequence areas of interest in the genome identified through GWAS. This, in turn, allows them to identify the **proteins** or **regulatory RNAs** encoded by those regions. The Roche Genome Sequencer FLX has computing power to sequence a length of DNA equivalent to more than 16,000 average-length human genes in less than a day.

Alex Stewart, manager of the Ruddy Centre, is helping to build the knowledge base of cardiovascular genetics with high-throughput technology, international collaboration and a steady stream of new discoveries. He is seen here with some of the advanced equipment that makes this work possible: Affymetrix Gene Chips and the Roche Genome Sequencer FLX.

(Dr. Robert Roberts: Towards Personalized Prevention and Treatment, continued)

from atherosclerosis, high cholesterol, diabetes, obesity and other conditions. A comprehensive understanding of the genetic underpinnings of risk for heart disease is expected to provide numerous benefits to patients.

"One will be improved prevention of heart disease," said Dr. Roberts. "Over the past 50 or so years, the data has been pretty consistent that somewhere around 50 per cent of heart disease is due to genetic predisposition. If we're going to really have comprehensive prevention efforts, we need to know both the lifestyle and genetic risk factors. So, for the genetic part of that, we need to find the genes."

"My ideal wellness centre could be one where someone could be assessed for both conventional risk factors and genetic risk factors," he explained. "From there, we would get a report card that would tell us what to do next. People think that if a risk is genetic, why bother knowing about it unless you can do something to treat the genes directly, but you can respond to genetic risk factors without actually having to change the genes themselves. If a patient knows that he or she is at higher genetic risk for high cholesterol or high blood pressure, then you can try to treat those conditions earlier and more intensively."

"Another big thing for patients is the idea of personalized medicine: treating the right person at the right time with the right drug. Any of these genetic variations are potentially targets for new drugs. Also, this genetic information lets us delve into what we call pharmacogenomics, the right dose of a drug for individual patients," continued Dr. Roberts.

"Pharmacogenomics is something that's affecting clinical practice today, using genetic information we've only recently acquired. We know that patients with a variation in a gene called *KIF6* need a higher dose of statins to reduce their blood cholesterol levels. Patients with variations in two other genes need lower doses of warfarin, to prevent dangerous bleeding. Whether or not a patient responds to treatment with clopidogrel, which is widely used to prevent blood clots, depends on variations in a gene that determines how the drug is broken down in the body. How patients respond to any drug is greatly influenced by their genes, and that's information we can adapt rapidly to improve patient care," he elaborated.

Dr. Roberts still hears feedback from people who suggest that the research community would get more of a payoff by focusing on lifestyle and environmental factors—like smoking, diet and exercise—instead of looking for genetic risk factors. The problem with that, he explained, is that a person's genes and lifestyle choices are inexorably intertwined.

"People talk about genetics and environment like they're two different issues, but the concept of separating the two has been dead for a long time," he said. "If you have a gene that predisposes you to high cholesterol but you're a strict vegetarian, you probably will never see the bad effects of that gene. Genes don't mean anything unless they interact with the environment, and the environment doesn't mean anything unless it interacts with the genes."

"Some people have a much tougher time quitting smoking than others, and there's already evidence that there's a genetic component. Some people can eat 1,200 calories a day and put on weight, and some can eat 3,000 calories a day and not gain weight," he added. "There's clearly a genetic component there. I would say, why would you concentrate on just a percentage of the population whose risk you can manipulate with lifestyle changes and not worry about those who clearly need something more?"

> "Why would you concentrate on just a percentage of the population whose risk you can manipulate with lifestyle changes and not worry about those who clearly need something more?"
>
> – Dr. Robert Roberts, President and CEO, UOHI

In the five years since the Ruddy Centre's inception, results from large-scale GWASs have been accruing at a rapid rate. Dr. Roberts now finds himself fielding more questions about when the public can realistically expect to see these discoveries translating into new treatments.

"You now have people saying, 'Well, you know about all these genes, but what have you done in terms of drug development?' I have to answer, please give us some time—this is very new technology and very new information," he said. "The mechanisms by which blood cholesterol influences heart disease were explained starting in the 1950s, but the first drug to treat high cholesterol was only available in the mid-1980s. There's always a large gap between the basic research and when you can bring new drugs to the bedside."

Statins came from a long discovery process that began with the discovery of a rare gene that caused familial high cholesterol, and involved understanding how cholesterol is made in the body and then how to inhibit that process. Any drugs targeting common genetic variations will come from a similar, gradual discovery process, but should result in treatments that are tailored to the genetics driving individual patients' disease, concluded Dr. Roberts.

Dr. Robert Roberts

" For research and for the Heart Institute in general, I think the Centre has been a major windfall, and it's been one of the best integrators for bringing people together in our research program—bench scientists, cardiologists, imaging experts, statisticians."

- President and Chief Executive Officer, University of Ottawa Heart Institute
- Director, Ruddy Canadian Cardiovascular Genetics Centre, University of Ottawa Heart Institute
- Internationally renowned cardiologist, cofounder of the field of molecular cardiology, an acclaimed leader in genetics and molecular biology of cardiovascular disease
- Regarded as one of the world's 50 most cited authors; named among America's Top Ten Doctors 13 consecutive years
- Recipient of the McLaughlin Medal from the Royal Society of Canada
- Recipient of the Distinguished Scientist Award from the American College of Cardiology and the Award of Meritorious Achievement from the American Heart Association
- Research Interests: Genetics and molecular biology of cardiovascular disease

MORE INFORMATION ONLINE

Visit the Cardiovascular Genetics Program:

www.ottawaheart.ca/research_discovery/cardiovascular_genetics.htm

Five Years of Discovery at the Ruddy Centre

Major Discoveries at the Ruddy Centre 2005–2010

- Variations in an area of the genome called chromosome 9p21 can increase the risk of heart disease by up to 40 per cent, independent of other known risk factors, such as high cholesterol and diabetes.
- These variations on chromosome 9p21 likely increase the extent of atherosclerosis, leading to earlier development of clogged blood vessels.
- Variations in a gene called KIF6 are not associated with the development of coronary artery disease, as had been proposed in earlier studies.
- Variations in a gene called ACSL5 influence a person's ability to lose weight by restricting caloric intake.
- International consortia with Ruddy Centre participation have found that variations in a gene called ARL15 and in an area of the genome called chromosome 12q24 also increase the risk of heart disease—the function of these areas of the genome are now being explored.
- Some cases of atrial fibrillation can be caused by somatic mutations in a gene called GJA5. Somatic mutations are not inherited but instead occur during fetal development.

Although scientists have estimated that 50 per cent of the susceptibility to heart disease is genetic, identification of these genetic factors has until recently remained elusive. Most genes discovered to date that influence the risk of heart disease have been identified through a targeted process, using families known to be at high risk and drilling down through their DNA to find the single genes responsible.

But high-risk single gene mutations account for a very small minority of cases of cardiovascular disease in the general population. "In the vast majority of diseases, many genes each contribute a small amount to risk, and discovery studies need to be very large and unbiased. It's a bit like panning for gold, and we need to sift through a lot of genetic material," said Alexandre Stewart, manager of the Ruddy Canadian Cardiovascular Genetics Centre.

In genetics research, this panning for gold is done through **genome-wide association studies (GWAS)**, which look for gene variations called **single-nucleotide polymorphisms (SNPs)**—changes in single units of DNA. SNPs are thought to largely account for the difference in susceptibility to various diseases observed among individuals in the general population.

In GWAS, SNPs found in a large group of people with heart disease are compared to those in a group of healthy people. SNPs occurring with a much higher frequency in the disease population are thought to be associated with risk. This assumption is then tested in several other populations to confirm the findings.

In the five years since its inception, the Ruddy Centre and its international collaborators have used GWAS to identify several important variations in the genome that contribute to an increased risk of cardiovascular disease, and have begun to understand how these variations influence risk at the molecular level.

(continued on page 5)

(Five Years of Discovery at the Ruddy Centre, continued)

Early Success

The Centre's first big win in cardiovascular GWAS came in 2007, with the discovery of a risk locus on chromosome 9p21, which is the strongest genetic predictor of early heart attack discovered to date (*Science*, June 8, 2007). A risk locus is an area of DNA in which variations in the genetic code are known to influence an individual's risk of a specific disease.

In a large GWAS of patients from the Ottawa Heart Study, validated in additional patients from the United States and Denmark to encompass more than 23,000 participants altogether, Heart Institute researchers led by Dr. Ruth McPherson identified two SNPs within the 9p21 region that significantly raised the risk of heart disease. The level of increased risk depends on the number of copies of either SNP: People with one copy have a 15 to 20 per cent increase in risk of heart disease, while people with two copies have a 30 to 40 per cent increase in risk.

This increased risk is independent of other known risk factors for heart disease, including blood cholesterol levels, high blood pressure and diabetes. "Until we discovered 9p21, we didn't know of another mechanism operating that makes the development of atherosclerosis more likely. We can't prevent something we don't know about, which is why this type of genetic research is so important," said Dr. Robert Roberts, Director of the Ruddy Centre and Heart Institute President and CEO.

The contribution of 9p21 to risk was not found to be evenly spread across races. While the SNPs significantly increase the risk of heart disease in Caucasians or Asians, they do not significantly affect risk in African-Americans, highlighting the importance of validating GWAS results in diverse populations.

Interestingly, the 9p21 risk locus does not encode a protein coding gene or **functional RNAs**. This result was not unexpected, explained Stewart. "Most of the mutations that exist in the genome are not in exons—the pieces of **DNA** that actually code for protein coding genes. Instead, they may alter **splicing**, or the levels of **gene expression**, or when genes are expressed. There's so much more to the genome than the protein coding genes," which is why it's important to scan the entire genome with GWAS, he explained.

Noncoding but Not Nonfunctional

Since 2007, Heart Institute scientists have begun to unravel how the SNPs on 9p21 contribute to the development of heart disease. In the paper first announcing the discovery of 9p21, the researchers suggested that it may somehow increase the extent of atherosclerosis, leading to earlier development of clogged blood vessels. In a follow-up study published in 2009 (*Arteriosclerosis, Thrombosis, and Vascular Biology*, October 2009), Institute researchers placed another piece of the puzzle by showing that the 9p21 risk **alleles** overlap a newly discovered **non-protein coding RNA** called ANRIL. Noncoding RNAs don't code for proteins, but can regulate the expression of protein-coding genes through various molecular mechanisms.

In these follow-up studies, the researchers discovered several variants of ANRIL in patients from the Ottawa Heart Study, and the activity of these variants was influenced by the SNPs on 9p21. Patients with the risk alleles had increased expression of the short variants of ANRIL and decreased expression of a long variant.

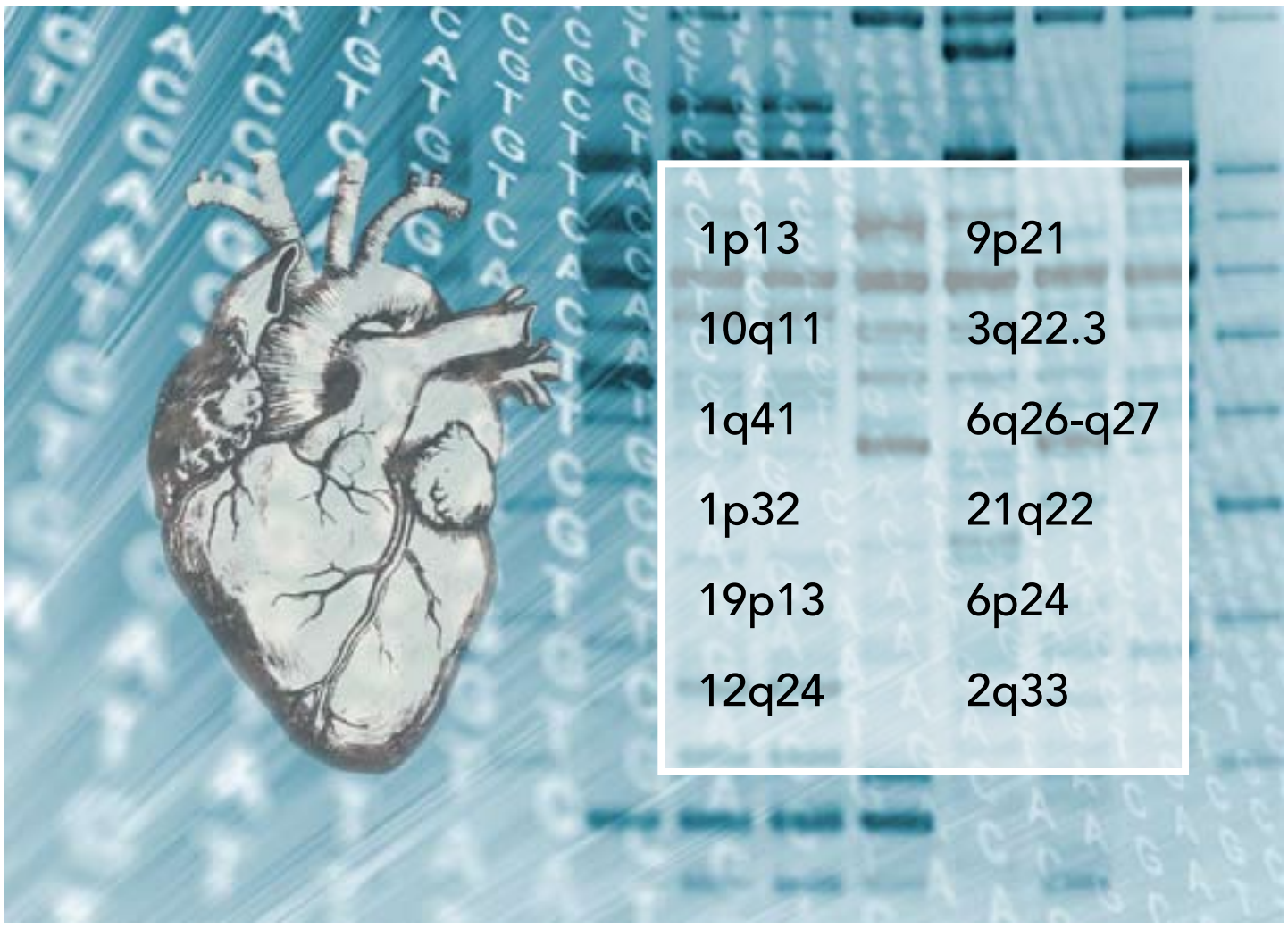
The researchers proposed that the SNPs may act as some sort of molecular switch that changes the expression levels of the two lengths of ANRIL **variants**. Alterations in ANRIL may in turn change the regulation of the cell cycle, affecting the proliferation and migration of smooth muscle cells, both of which play a role in atherosclerosis.

While the mechanisms of 9p21's influence on coronary heart disease continue to be explored, additional information on the end result is also accumulating. In a follow-up study presented at the Scientific Sessions of the American Heart Association in 2009 and slated for publication later in 2010, Heart Institute researchers report that 9p21 can be used to predict the severity of coronary heart disease in addition to the risk of disease. The study included participation from both genetics researchers and interventional cardiologists, who diagnosed the severity of heart disease in approximately 1,600 patients while unaware of their genetic status. Individuals with disease in all three coronary vessels of the heart (triple vessel disease) are more likely to have two copies of 9p21.

"This work satisfies one of the major goals of the Centre, which is finding risk factors that will predict severity of disease," said Stewart. "With that knowledge, we can be a little more aggressive with patients known to be at high genetic risk, in terms of promoting dietary change, smoking cessation and other beneficial lifestyle changes."

Revisiting Results

Another important advantage of large GWAS studies, explained Stewart, is their ability not only to discover new genes, "but to refute prior findings that have been made in a somewhat haphazard way."



To date, the dozen genes listed above have been shown to be associated with heart disease. These include 9p21, discovered by Heart Institute scientists. A given gene may have multiple variants, some protective and some risk-elevating. Several more genes, identified with Heart Institute collaboration, are set to be published in the coming months.

The Genetics Centre recently did this for a gene called KIF6. The significance of KIF6 to coronary artery disease has been inconclusive—some previous studies have suggested that SNP within KIF6 associates with coronary disease, and some have not found such an association. However, a commercial test is already marketed using KIF6 as a predictor of disease risk.

In a Heart Institute study published in 2009, researchers found no association between SNPs within KIF6 and coronary artery disease (*Journal of the American College of Cardiology*, April 21, 2009). The study looked at more than 1,600 patients with early onset of the disease, recruited from the Institute's Lipid Clinic, and more than 1,400 control patients from the Ottawa community. Several other published investigations have confirmed the findings at the Heart Institute. Additional data taken from the Women's Health Study from the United States National Institutes of Health suggest that variations in KIF6 may associate with acute heart attack but not chronic heart disease, underscoring the complexity of cardiovascular illness.

Ruddy Centre researchers have also participated in initiatives to increase the quality of reporting of GWAS results by enhancing transparency of the design, conduct and data analysis of these studies. The STrengthening the REporting of Genetic Association (STREGA) studies statement, published in 2009 (*Annals of Internal Medicine*, February 3, 2009), was designed to improve the quality of research papers in the field, which in turn will help researchers better evaluate the strengths and flaws of newly published GWAS.

Influencing Weight, Influencing the Heart

The Ruddy Centre's search for genetic variations that influence heart disease also encompasses variations contributing to conditions that directly influence cardiovascular disease risk, such as obesity. In a recent study from the Centre, researchers identified an SNP associated with diet-induced weight loss (*FASEB Journal*, June 2009).

People with the SNP located in a gene called ACSL5 produced more of the ACSL5 protein in skeletal muscle and were more likely to be able to lose weight by restricting caloric intake. ACSL5 is part of a family of proteins that help muscle take up free fatty acids, the form in which the body burns fats for energy.

Interestingly, the researchers proposed that natural selection has likely made this SNP less common in the general population, as people who are less prone to weight loss would be more likely to survive a famine by storing fat rather than burning it for energy.

Shared Effort, Greater Knowledge

Much of the Centre's recent work has involved international collaborative groups who have pooled their resources and genetic samples to allow for even larger and more robust studies of the genome and risk of cardiovascular disease.

At the end of 2009, the international Genetic Investigation of Anthropometric Traits (GIANT) consortium, including the participation of the Ruddy Centre, identified SNPs in a gene called ARL15, which influences blood levels of a protein called adiponectin (PLoS *Genetics*, December 2009). Adiponectin is known to decrease the risk of both atherosclerosis and diabetes.

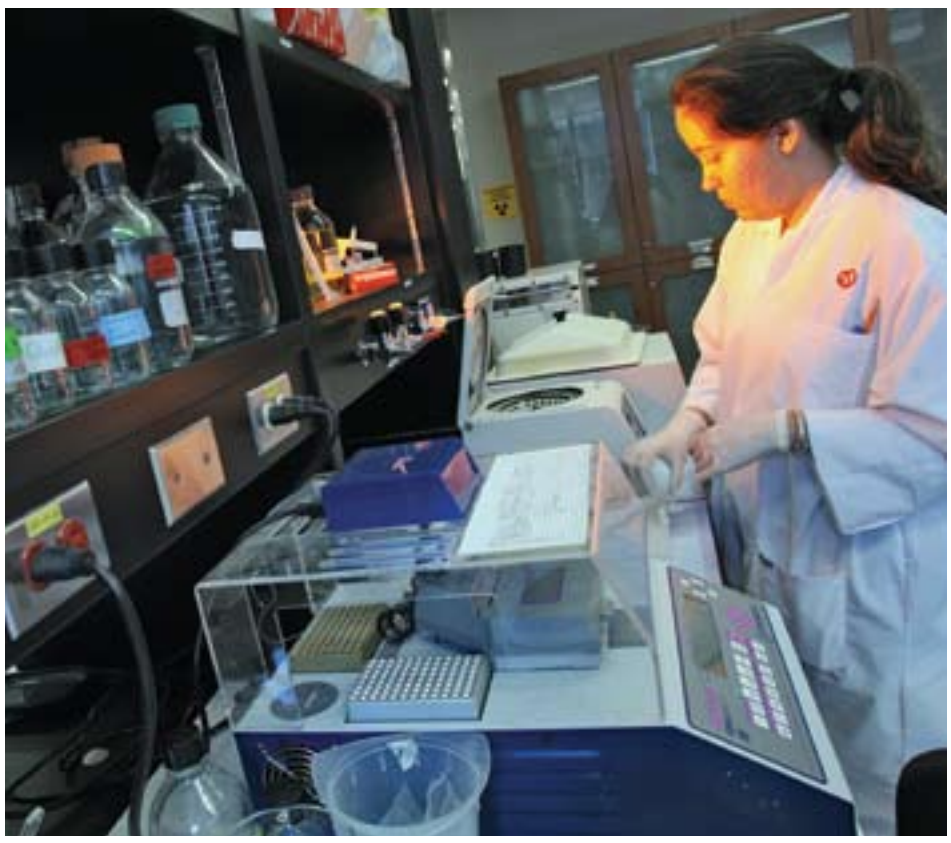
One of the SNPs that lowered adiponectin levels was also associated with an increased risk of coronary heart disease in seven different cohorts of patients tested for the study. Although the function of ARL15 is currently unknown, the GIANT researchers speculated that it may play a role in insulin resistance or in the transportation of adiponectin through the body.

(continued on page 6)

(Five Years of Discovery at the Ruddy Centre, continued)

Also in 2009, another international consortium with Ruddy Centre participation, the HaemGen consortium, published the largest genome-wide association study looking at differences in blood cell counts based on SNPs, including validation in three separate populations (*Nature Genetics*, November 2009). Both the number and composition of blood cells in the body are highly influenced by heredity, and variations from the normal ranges of blood cells indicate risk for many diseases, including heart disease and cancer.

The HaemGen study identified a new risk locus on chromosome 12q24 associated with both coronary artery disease and heart attack. Interestingly, genetic variation at 12q24 has also been linked to diabetes, high blood pressure and celiac disease (an autoimmune disorder), making it a true pleiotropic gene—one gene that has many different effects on the body.



Genetic research isn't all about sopisticated technology. Appropriate participants must be recruited for the research and blood samples taken. DNA is then extracted from the white blood cells and is cloned (shown here) using a process called polymerase chain reaction to enrich the sample. The genetic material is then ready for sequencing, genotyping, or inserting in other cells to develop disease models.

Deep Sequencing

Not all research at the Ruddy Centre uses GWAS to uncover the genetic basis of disease. Room still exists for targeted studies when researchers have an inkling of what genes may contribute to a specific disease process. Based on earlier work in mice, Dr. Michael Gollob and his colleagues homed in on somatic mutations (mutations that are not inherited but instead occur spontaneously during development) in a gene called GJA5 as a potential cause of atrial fibrillation. GJA5 encodes a protein called connexin 40, which normally helps carry the electrical signal from atrial cell to atrial cell.

In work published in 2006, the researchers sequenced the complete GJA5 gene in 15 patients with early onset atrial fibrillation (*New England Journal of Medicine*, June 22, 2006). They found that four patients had mutations in their heart tissue that affected the function of connexin 40 and, therefore, the heart's rhythm. Three of these were somatic mutations that arose during fetal development—the mutations were not found in other tissues of the body.

While it has been known that gene mutations causing heart disease can be inherited and lead to high risk within families, until this study, it had not been well-established that mutations during in utero development can also trigger disease.

Although such somatic mutations are likely uncommon in the general population, the findings show that atrial fibrillation can have a genetic component that could possibly be targeted with new drugs, whereas doctors have normally considered the disease to arise spontaneously from physiologic causes.

The Centre is currently embarking on complete genetic sequencing of several areas of the genome associated with increased risk of cardiovascular disease that were discovered through GWAS performed by the CARDIoGRAM consortium, and beginning new studies looking for genetic variations associated with high blood pressure.

Genes as a Key to Treatment

The long-term motivation for all research into the genetic causes of disease is the hope of more personalized medical care. "The best health care should be targeted care, because we're all different at the genetic level, and we won't all respond the same to treatment in the same way," said Stewart.

The Centre is currently working with partners in the biotechnology industry to tailor new drugs to patients who are most likely to benefit from those drugs. "We're definitely looking to move these discoveries from the bench to the bedside to address the most urgent needs of our patients," he continued.

One somewhat frustrating aspect of using GWAS discoveries for drug development is that the process is reversed from normal targeted drug development, which is based on a specific gene already known to cause disease. In GWAS, "you find the genetic targets before you know what they do, so getting drugs to market will take a bit longer," explained Stewart.

However, the researchers at the Ruddy Centre believe that the eventual payoff in improved care with reduced costs will make the wait worthwhile.

Uncovering the Genetic Roots of Salt Sensitivity in High Blood Pressure

Dr. Frans Leenen

When Dr. Frans Leenen first started researching hypertension, or high blood pressure, he would have stared at you in disbelief if you'd suggested what a central role genetic technology would come to play in his research.

Today, however, the Director of the Hypertension Clinic and Hypertension Research at the University of Ottawa Heart Institute is on closer terms than he'd ever imagined with geneticists and genetic technologies. And it's all because he wanted to know more about why some people are sensitive to salt in developing high blood pressure, while others aren't at all.

The majority of people over the age of 60 develop high blood pressure. But many people develop it early. In many of these cases, there is a genetic basis for the high blood pressure—in other words, it runs in the family. About half of these people are what is known as "salt sensitive"—they react strongly to salt found in the food we eat.

Dr. Leenen is conducting a study, funded by the Canadian Institutes of Health Research, that is looking for **genetic variants** that could contribute to salt sensitivity. In the first phase, he is establishing a **phenotype**, or a picture of the relationship between salt and blood pressure, of about 500 people who are hypertensive. The researchers are collecting blood and urine samples as well as 24-hour blood pressure readings, while participants eat a low-salt diet for four weeks, followed by a high-salt diet for four more weeks.

In the second phase of the study, Dr. Leenen will compare these and other people with hypertension with 1,500 healthy control subjects, older adults who have not developed high blood pressure. His co-investigators, Alex Stewart of the Ruddy Canadian Cardiovascular Genetics Centre and Fréderique Tesson of the University of Ottawa, will then analyze the **genotype**, using the technology of the Ruddy Centre to search for genetic mutations that may characterize salt sensitivity.

"We hope to see that the gene variants we find in the phase I population will be seen in a higher percentage in the phase II hypertensive population, compared to the population of normal-blood-pressure controls," he said.

"This project is clearly an example of good collaboration," Dr. Leenen added. "We could not do the study alone, and the Ruddy Centre could not do the study alone. Doing good phenotyping is a major aspect of good genetic research."

The phenotyping being done through this study will be of higher quality than most studies to date because, Dr. Leenen said, they are basing it on around-the-clock blood pressure readings and urine collection, rather than on one or two samples of either taken during an office visit. By providing a more rigorously defined pool of samples, this should make Stewart and Tesson's work that much easier.

If Dr. Leenen and his co-investigators are able to identify **genetic markers** for salt sensitivity, physicians will be able to screen for these markers, ideally using a simple blood test. But that all depends on how many genetic mutations are involved—the more mutations, the more complicated it is to screen for them. But Dr. Leenen is optimistic. "My instinct says that we will find a few only," he said.

Dr. Leenen also hopes to learn more about the mechanisms by which people are sensitive to salt.

"There is a lot we know about the 'how', but we don't know enough about the 'why,'" he said. "That is why genetic studies are so important." Salt sensitivity, for instance, has commonly been blamed on the kidneys. Dr. Leenen's animal studies suggest that the brain is more important—that when people who are salt sensitive consume too much salt, the salt content of their brain increases, leading to high blood pressure. The process whereby this happens, though, is complex, involving a cascade of actions and reactions.

We don't know why salt levels in the brain go up in some people and why that translates to higher blood pressure. Dr. Leenen hopes that learning more about the underlying mechanisms at each stage of the cascade will present new targets for therapies—molecules and **proteins**—where intervention could help prevent and treat hypertension.

Public health policies to cut the salt in food are necessary, added Dr. Leenen. But the policies will take a long time to have an impact and they are unlikely to bring salt in food down to levels where the salt no longer affects blood pressure. Being able to screen people at risk for hypertension would mean being able to intervene earlier to prevent the condition from developing or treating it early.

That is the direction Dr. Leenen believes hypertension research is going. In the past, he explained, it was all about developing medications and treatment. Today, with most hypertension in Ontario identified and well-managed, it is time to focus more on prevention. And genetic research will help in achieving this population-health goal.

MORE INFORMATION ONLINE

Visit Dr. Frans Leenen's Lab Page:

www.ottawaheart.ca/research_discovery/leenen-laboratory.htm

The Genetics of Coronary Artery Disease and Its Metabolic Risk Factors


Dr. Ruth McPherson

As an endocrinologist and Director of the Lipid Clinic at the University of Ottawa Heart Institute, Dr. Ruth McPherson sees firsthand how metabolic risk factors such as high cholesterol and obesity, can lead to coronary artery disease (CAD). As Director of the Atherogenomics Laboratory, she is studying the genetics of CAD and its contributing risk factors, including plasma lipid levels and obesity.

Dr. McPherson's genetics research has focused on two main areas: the genetic origins of CAD and the genetic variants that contribute to obesity. Much of this research is carried out through **genome-wide association studies,** which require the processing of large numbers of samples and large amounts of data. The presence of the Ruddy Canadian Cardiovascular Genetics Centre has greatly facilitated her research in this area and has contributed to the identification of novel risk and protective factors.

The Centre's high-throughput capacity has given Heart Institute researchers the ability to do large scale **genotyping** and **sequencing** on-site rather than outsourcing it, as was previously done. This capacity has also helped put the Heart Institute on an international footing in the world of genetics research.

"Before the Ruddy Centre opened, we could only do very conventional genotyping and sequencing," said Dr. Ruth McPherson. "If we wanted to do more extensive work, we had to collaborate with investigators in the United States. Now, we can accomplish most of the sophisticated laboratory analyses by ourselves."

Both rare and common **genetic variants** may account for more than half of people's susceptibility to CAD. Dr. McPherson's research into CAD focuses on identifying those variants. The research has been conducted through the ongoing Ottawa Heart Study, which compares the genomes of 3,500 younger Canadians with CAD with the genomes of more than 2,500 elderly patients who have no signs of CAD.

In 2007, her research led to the discovery of an **allele** on the 9p21 chromosome that is a genetic risk factor for heart disease. The 9p21 risk allele increases the likelihood of developing premature atherosclerosis, or narrowing of the arteries. Importantly, 9p21 appears to increase risk for CAD independently of other risk factors, such as smoking, diabetes, high blood pressure or high cholesterol. About 25 per cent of the population carries two copies of these risk alleles, making their risk of early onset CAD twice as high as that of individuals without these variants.

The identification of 9p21 is regarded as one of the most important discoveries in the genetics of cardiovascular disease. Researchers throughout the world are now focusing on 9p21, trying to find out how it works. By understanding how it functions, scientists may one day be able to develop new drugs for the prevention or treatment of CAD. Dr. McPherson's laboratory has recently made important advances in unravelling the link between 9p21 and CAD.

Close to 25 novel genetic variants linked to heart disease risk have now been identified as part of a collaboration amongst numerous laboratories around the world, including the scientists in the Ruddy Cardiovascular Genetics Centre.

The other major focus for Dr. McPherson's research, in collaboration with Dr. Robert Dent in the Weight Management Clinic at the Ottawa Hospital and Mary-Ellen Harper at the University of Ottawa, is on the genetics of body weight regulation. Obesity is a major risk for type 2 diabetes, which is one of the strongest risk factors for developing heart disease. Obesity also increases the likelihood of developing high blood pressure and cholesterol abnormalities. Dr. McPherson is trying to identify genes implicated in obesity by comparing the genomes of extremely obese and extremely lean individuals.

Through the Thin Gene Study, Dr. McPherson and her colleagues sequenced 58 candidate genes in 1,000 ultralean and obese individuals. As a result, they were able to identify several novel rare genetic variants that could potentially contribute to obesity. "This study demonstrated that at least 5 to 10 per cent of obese people have variations in genes that put them at high risk for obesity," she said. "These are the individuals who often require bariatric surgery to achieve and maintain a normal weight. For many people with major genetic risk factors for obesity, diet and lifestyle just aren't enough."

Dr. McPherson is now completing a large genome-wide association study of obesity and leanness using the Ruddy Centre's Affymetrix 6.0 **gene chip** to identify common genetic variants contributing to body weight regulation.

Dr. McPherson believes the day is close when her genetic research will be able to be applied in a clinical setting. But, she added, because of the complex nature of the genetics of heart disease and the multiple genes that are involved, clinical application needs to be approached with caution.

"This is certainly a goal in some of the work we're doing, to determine how much better we can predict future risk of heart disease in young, otherwise healthy people, incorporating both conventional risk factors and data from common genetic variants that have been linked to heart disease," she said. "Genetic analyses are not currently part of routine screening in CAD prevention clinics. However, this may change as our ongoing research identifies even more genetic variants linked to heart disease risk.

Translating Genetics into Better Patient Care


Dr. Michael Gollob

> "Genetics is now out of the realm of pure research. We can use genetics now to make a diagnosis, determine the prognosis for the patient and make a treatment plan."
>
> – Dr. Micheal Gollob, Director of the Genetics of Cardiac Arrhythmias Research Laboratory, UOHI

Genetics wasn't part of the training for most cardiologists practicing today—mainly because cardiac genetics really didn't exist as a field. After all, the first gene implicated in heart disease—the gene for hypertrophic cardiomyopathy—wasn't found until 1990. And it wasn't until 2006 that Dr. Michael Gollob was involved in finding a novel gene for atrial fibrillation—the most common kind of arrhythmia affecting people.

On top of this, most cardiac disease is not caused by a single "bad" gene, except in one key area—arrhythmia. Some forms of arrhythmia, or irregular heartbeat, affect young people. And often, the first they or their families know of it is when someone close to them suffers a sudden cardiac death.

Genetic testing can detect a risk of fatal arrhythmia before it kills and allow people to take preventive measures. But their physicians need to know that it is available, when to use it and, just as importantly, when not to use it, explained Dr. Gollob, Director of the Genetics of Cardiac Arrhythmias Research Laboratory and of the Inherited Arrhythmia Clinic at the University of Ottawa Heart Institute.

"Having genetic information improves care for our patients and their families," he said. "But you must be selective and mindful of when to go to genetic testing."

Dr. Gollob is the leader of a group of Canadian cardiologists who have developed the first guidelines in the world for genetic testing in the clinical evaluation of inherited arrhythmias associated with sudden cardiac death, on behalf of the Canadian Cardiovascular Society and the Canadian Heart Rhythm Society.

"Genetics is now out of the realm of pure research. We can use genetics now to make a diagnosis, determine the prognosis for the patient and make a treatment plan," said Dr. Gollob. "We know enough now to apply this information in the care of patients in clinical practice. But many cardiologists simply don't know how—it's just not part of their practice."

The guidelines, which will be published later in 2010, are intended to help physicians. But health policy makers are also the target, indicated Dr. Gollob. "Genetic testing and genetic information are now part of the standard of care. They are as important as an MRI or an angiogram, as important as any other cardiac test. We're lucky; genetic testing is funded in Ontario if we think it is needed. But some other provinces don't fund it."

Atrial fibrillation, the primary focus of Dr. Gollob's research, affects 250,000 Canadians and is forecast to affect some 20 million Americans by 2050. It can lead to stroke and creates a burden on both patients and health systems. Current treatments—generally medication or a procedure called cardiac ablation—are not very effective, Dr. Gollob said, in part because they don't target the specific cellular mechanisms that cause atrial fibrillation. Complicating matters further, there is no one specific mechanism involved, as Dr. Gollob's research is making clear. This underlying genetic diversity makes treating the disease even more challenging.

The Genetics of Cardiac Arrhythmias Laboratory is the only facility in the country involved in genetic research on sudden cardiac death and other arrhythmias. Using the technology available to them at the Ruddy Canadian Cardiovascular Genetics Centre, Dr. Gollob and his colleagues evaluate novel genes that might be implicated in different kinds of arrhythmias. By conducting research into the genetic mechanisms that lead to arrhythmias, he hopes to discover specific genes and **proteins** that cause diseases such as atrial fibrillation. These genes and proteins could then be used as targets for drug development. Identifying the genetic mechanisms of disease would allow for the unravelling of specific molecular

(continued on page 8)

Uncovering the Genetic Mysteries of Heart Development

When babies are born, their hearts are ready to pump blood throughout their bodies thanks to an exquisite developmental trajectory in which specific **genes** tell fetal cells to divide and differentiate into various kinds of heart cells. About two weeks after birth, however, heart cells stop dividing and increasing in number. From then on, heart cells grow in volume only.

That strategy makes sense, said Heart Institute researcher Patrick Burgon. It allows cells to grow larger when the heart needs to work harder—in a marathon runner, for example, or someone whose heart isn't working effectively—and shrink back down again if the heart is able to ease off a bit, say, if that marathon runner turns into an occasional slow jogger instead.

"There's a constant fine-tuning that goes on, depending on the demand that's placed on your heart," said Burgon.

There's one exception to that rule. When the heart suffers damage, such as from a heart attack, some of those fetal genes are reactivated. The presence of these fetal genes is one of the classic signs of a sick heart, Burgon said, and their detection—in particular, detection of a marker called ANF, a hormone that was discovered by Adolfo de Bold, the founding Director of Research at the Heart Institute—lets physicians start treatment early to prevent further damage.

Some people believe the heart is trying to grow new cells to repair the damage. But, Burgon said, "I don't think the heart's that smart." His view is that, because the heart is sick, **gene expression** is not well-controlled. But if researchers could discover how to take advantage of this reactivation, to encourage new cell creation and division after heart attack, then they could figure out how to regenerate healthy heart tissue—literally, how to get the heart to grow those new cells it needs.

These are exciting possibilities. Burgon, who is a member of the Cardiovascular Endocrinology Group at the Heart Institute, is contributing his own piece of the puzzle, through his study of a transcription factor (a protein that attaches, or binds, to DNA to control the transmission of genetic information). He has discovered a transcription factor called MLIP, or muscle lamin interacting protein. MLIP is completely unique—no even remotely similar gene shows up in


Patrick Burgon

all of the libraries of genomes, whether human or animal.

"At times, I thought we were fools for chasing it," said Burgon. "But now, as our findings accumulate, I think we're on to something."

His challenge is to understand what MLIP does and how it works. He needs to find out where it is, what tissues it shows up in and at what point in human development it occurred. He believes its role will be in early development. The discovery has exciting implications. For instance, said Burgon, the gene could be used to differentiate stem cells into precursor heart cells, to repair damage and regenerate healthy heart cells. Or it could be used to stabilize damaged tissue to prevent further damage and regain some function. The existence of MLIP provides potentially new therapeutic strategies and targets for new drug development.

Burgon is also looking closely at the hearts of mice just before birth and at one, three, five, seven, and ten days and five weeks old (when a mouse is considered adult) to map the genes that are present at all of these different stages. He believes that a transitional program exists to take the fetal heart to an adult heart, and he hopes that the secrets of this transition will emerge from his analysis.

"It's very CPU intensive," he said of the data-processing power required. "We're trying to identify genes that are important, but we're more interested in pathways than in single genes." This, he said, makes the already complicated job of searching for genes just that much more complex.

The Ruddy Centre has been central to his work. "It's been a huge benefit for us—all our **sequencing**, our arrays–just the convenience of having it here. It's a huge factor." In fact, the Ruddy Centre is part of what convinced this rising star to choose the Heart Institute after completing two post-doctoral fellowships at Harvard.

"I just felt there was so much potential here—the resources are amazing."

MORE INFORMATION ONLINE

Visit Patrick Burgon's Lab Page:

www.ottawaheart.ca/research_discovery/molecular-signaling-laboratory.htm

Heart Institute Research that Extends Far Beyond Heart Disease


Heidi McBride

Their origins lie in the distant past, but they play a hugely important role in present-day disease, including heart disease.

Mitochondria are small organelles, or compartments, found in cells. They are believed to have originated in ancient bacteria. They may be small, but their role is vital. They take the oxygen we breathe and convert it into the energy cells need to carry out their functions. When cells don't get the oxygen they need, they shut down.

In a heart attack, explained Heidi McBride, oxygen is cut off to the cells of the heart. Deprived of oxygen, the cells start to release free radicals, highly reactive atoms or molecules which are associated with oxidative damage. This damage causes cells to age and, eventually, die. "If we can figure out how to hold the mitochondria steady during times of low oxygen, we could then develop drugs that would help prevent cell damage and death," she said.

McBride, Principle Investigator of the Intracellular Dynamics Lab at the University of Ottawa Heart Institute, is a world-leading researcher in the area of mitochondrial function, contributing new knowledge about how these "energy powerhouses" work. Her lab was among the first to identify a novel pathway whereby mitochondria deliver some of their contents to another cellular organelle called the peroxisome, which has much in common with mitochondria. She is now investigating why these pathways exist and how they are regulated. She also is involved in studying mitochondrial fusion and division, both of which have implications in heart disease and stroke.

Her current research is using the power of genetics to investigate how genes affect mitochondrial function. She is looking at 18,000 **genes**—nearly all of the roughly 20,000 genes in the **human genome**—to discover which genes affect mitochondrial function. It's a big job, made possible because of a robotic platform that can conduct high-speed screening of large numbers of genes. The main job of genes is to produce **proteins**, which are required for cell function. One gene at a time, the platform puts in a sequence that inactivates protein production. That way, McBride will be able to tell how inactivating each gene in turn affects mitochondria.

The platform is located at the Children's Hospital of Eastern Ontario Research Institute, and is unique in the world. McBride's colleague, Dr. Robert Screaton, established the technology and is working with her on this project. The data derived from use of the platform will be analyzed in multiple labs, including at the Heart Institute.

This is the first research of its kind, screening human genes to see how they affect mitochondrial shape and function. The knowledge that is gained from the research could help in the identification of targets for interventions to prevent or reduce the damage from heart attacks. And while Dr. McBride is looking primarily at heart cell function, her research is applicable to a wide range of diseases.

"I'm a very basic scientist," she said. "Even though you're working in the Heart Institute, you never know what you're going to come up with. The things we find in this lab are central to many diseases, like diabetes, heart disease and Parkinson's."

In diabetes, for instance, as a key metabolic sensor, the mitochondria play a role in the ability of beta cells in the pancreas to produce insulin; they also have a hand in the cell death that occurs in type 1 diabetes. Dr. Screaton is searching for new insights into the onset of type 1 diabetes and, in collaboration with Dr. McBride, will use the genome-wide screen to look closer at the mitochondria in this process. McBride is also collaborating with a group of neuroscientists to investigate the role of the mitochondria in Parkinson's disease. Almost all of the genes identified that carry mutations in Parkinson's patients encode proteins that interfere with mitochondrial function. Similar to heart disease, mutations in these genes lead to the accumulation of damaged mitochondria, which after a number of years, leads to premature cell death and the onset of symptoms for the patients.

"All of these diseases have in common that cells die, and mitochondria are central to cell death," Dr. McBride said. "If we can find the tools to make mitochondria happy, we can stop cell death and prevent heart attacks and other diseases."

MORE INFORMATION ONLINE

Visit Heidi McBride's Lab Page:

www.ottawaheart.ca/research_discovery/intracellular-dynamics-lab.htm

(Translating Genetics into Better Patient Care, continued)

pathways that could be intervened upon by treatment, even for patients in whom no specific genetic cause has been identified.

As well, Dr. Gollob added, there is more than one genetic pathway involved in arrhythmia. Understanding the different pathways would mean that it would be possible to determine which pathway is involved in an individual's arrhythmia and tailor treatment to target that particular pathway best.

It is this potential to help his patients that motivates Dr. Gollob. As a researcher and a clinician, he said, when his patients raise questions, he can go to his lab to look for answers, and, hopefully, find improved ways to care for these patients.

"I have a passion for asking why," he said. "And I want to try to answer the question myself. Clinicians have the unique opportunity to see the disease, and to follow it from the bedside to the cell and back again."

Advances in Science Create Demand for Genetics Services

As the Heart Institute's genetic counsellor, Julie Rutberg has seen her patient load double in the last few years with the increased availability of genetic screening tools. Her patients include those with heart disease as well as people whose family history puts them at heightened risk.

The past several years have seen an explosion in the identification of genes implicated in heart conditions—and that's made Julie Rutberg's life much busier.

Rutberg is a genetic counsellor at the University of Ottawa Heart Institute. Her role is to help patients with understanding and interpreting the results of genetic testing and what those results mean for them. But her patient load is not only getting bigger—she saw 65 patients in the first three months of 2010, double the number she saw just three or four years ago—it's also getting more diverse. She's seeing patients with different conditions—patients from the Adult Congenital Heart Disease Clinic, for instance, and young people over 18 who are no longer being seen at the local children's hospital—and many people who don't have a heart condition at all, but who are at risk based on their family history.

When a genetic heart condition is diagnosed, other family members can learn if they, too, are at risk. They can be tested to see if they have the gene for the condition, even before symptoms appear. That means that measures can be taken to help prevent or slow the progression of the condition. But it can also mean that people who consider themselves healthy all of a sudden have to start thinking of themselves in an entirely different way. And that can be difficult.

"We balance 'you are at risk' with not scaring them so much that they become anxiety ridden," said Rutberg. "They can decide they don't want to exercise, that they don't want to participate in life because they have something. We have to balance all their different needs."

Educating patients and encouraging them to refer their family members for testing is a delicate matter, Rutberg said. Every person is different and every family has different dynamics. Some patients simply tell their family members directly. Others who aren't as comfortable doing this may turn to "Aunt Mary," the family member who talks to everybody and knows what's going on with everybody, to spread the news. Rutberg's role, she said, is to help people figure out what's best for them and their family.

As well as family members, Rutberg has also identified family physicians as a target for education. She hasn't done anything to date—it's been word of mouth so far that has family physicians referring patients to her for testing—but she knows that it's growing as a priority.

> With each additional gene that is tied to a disorder, the chances of being able to detect that disorder go up.

One disorder with a genetic basis is hypertrophic cardiomyopathy, a condition that results in thickening of the heart muscle. The thickening makes it harder for blood to leave the heart, forcing the heart to work harder. Hypertrophic cardiomyopathy occurs in one in 500 people and many people can have it without knowing it.

In the past five years, it's become possible to test for the condition. And that, says Rutberg, has made a huge difference. When someone is diagnosed with the condition, family members can have a simple blood test to see if they have it too. Previously, the only way of detecting the condition was with echocardiograms every few years.

"We can't prevent the disease from happening," said Rutberg. "But if we can identify who's at risk, we can get them to change their lifestyles and take other steps to avoid complications."

As knowledge of genetics grows, so too does an awareness of just how complicated it can be. At one time, it was thought that one gene equalled one disorder. But that idea is "pretty much out the window now," said Rutberg. "It's multiple genes causing multiple disorders."

That can be a good thing, though. With each additional gene that is tied to a disorder, the chances of being able to detect that disorder go up. In 2002, for instance, no genes for another kind of cardiomyopathy–arrhythmogenic right ventricular cardiomyopathy, or ARVC–had been identified. Then there were two. Now there are five. And, with each additional gene, the percentage of people at risk who can be identified has gone up. Now, genetic testing can detect about 50 per cent of cases.

As well, Rutberg said, there's a greater understanding now that genes do not equal destiny. Different family members with the same gene can have completely different experiences. While family members can share the culprit gene, some members may have other, protective genes. And some members may experience triggers that activate the harmful gene, while others don't—perhaps something in the environment or a virus.

Rutberg anticipates that this is the next development in genetic medicine: a move from simply identifying people at risk to identifying other factors that make it more or less likely that those at risk will develop a condition. And, she anticipates, this will lead to the development of individualized treatment plans, based on an individual's specific profile—a **genotype/phenotype** correlation she calls it.

"That was always the Holy Grail of genetics," says Rutberg. "Now, as we've gotten more and more data as we test more people, we are getting closer to being able to make that correlation."

Cardiac genetic counsellors are rare—only four or five clinics in Canada have such a positions. But, as the number of genes tied to cardiac conditions increases, their role is becoming increasingly important.

"My role here is to be the genetic expert, so I can be a resource available to all the specialty areas within cardiology," she said.

MORE INFORMATION ONLINE

See the video "When knowing about heart disease brings relief":

www.ottawaheart.ca/about_us/making_a_difference_in_peoples_lives.htm

Cardiac Autopsy Guidelines Spreading across the Continent

Cardiac autopsy guidelines originally developed at the University of Ottawa Heart Institute are rolling out across the country—and south of the border as well.

The guidelines are used to help diagnose unexplained cardiac deaths among people under 40. Many of these deaths—which most often receive public attention when they occur in athletes under the age of 18—are due to cardiac arrythmias, or problems with the electronic functioning of the heart, that would not show up in a standard autopsy. These are often inherited conditions, carried in the genes we inherit from our parents. The cardiac autopsy guidelines specify a standard protocol for examination of the heart that is more extensive than that employed in a standard autopsy, to exclude conditions that may leave little evidence in the organ but that would not otherwise be detected. The guidelines also provide guidance to pathologists on tissue collection and storage to better enable genetic screening, known as a molecular autopsy.

When a sudden unexplained cardiac death occurs, using the cardiac autopsy guidelines means that grieving family members have a better chance of finding out what happened to their loved one. Even more important, if the death turns out to be due to an inherited condition, they can get tested themselves for the same condition and take precautionary measures should they also be at risk.

The guidelines were developed by the Heart Institute and the Chief Coroner's Office, and with the Children's Hospital of Eastern Ontario, Toronto's Hospital for Sick Children and The Ottawa Hospital; they have been in place in Ontario since 2008. Last summer, the Heart Institute's Dr. Martin Green spoke about the guidelines at a national conference of Canadian Chief Coroners. Attendees from all provinces and territories went home convinced of the utility of the guidelines in their own jurisdictions.

It's not easy, said the Heart Institute's genetic counsellor, Julie Rutberg, who was involved in the development of the guidelines. Each province and territory has its own way of operating their coroners' offices, and coroners have different qualifications. What is important, she said, is that all involved work together.

"They may show up looking somewhat different in different places, and that's OK," she said. "But cardiologist, geneticists and pathologists have to work together. No one specialty can do it alone."

Rutberg is also working with the National Association of Medical Examiners in the United States and with her fellow genetic counsellors there to promote the use of the guidelines there.

"We're happy this isn't just knowledge that's sitting in Ontario," she said. "It makes us feel like we had some leadership."

In Conversation

John Ruddy

Great ideas require great champions and support in order to come to fruition. The Ruddy Canadian Cardiovascular Genetics Centre was made possible in large part due to the generous support of John and Jennifer Ruddy, who contributed $5 million to help launch it in 2005.

The Ruddy's donation was complemented by an additional $1.7 million from several Ottawa-area donors. The "Founding Partners" of the Centre each contributed gifts of $500,000. These generous supporters included: The Harold Crabtree Foundation, Herb and Dorothy Nadolny, and business partner and friends Lyon and Dundi Sachs, the Vered and Besner families, and Cognos Inc. founder Michael Potter.

John Ruddy is an Ottawa business leader, philanthropist and former patient of the Heart Institute. In this interview, he talks about why he chose to support the Genetics Centre.

The Beat: You're involved with projects in health care, the arts, sports and more. How do you choose to get involved with something?

John Ruddy: I look at it on the basis of community building, and my choices are shaped by my personal experiences. It's really that simple. In this case, it wasn't just about the benefits of the specific research activity; it was about the ripple effect through the whole community, which is always a very positive thing.

The Beat: What got you involved in this project?

John Ruddy: Well, the Heart Institute and its terrific team of doctors, nurses and researchers were there for me and my parents when we needed them. So, I had a connection to the Heart Institute.

But I think what excited me was that, as you know, Dr. Roberts is a very compelling man. His enthusiasm is contagious and he obviously has a worldwide reputation in the field. I was strongly encouraged by his desire to really focus on this whole genetic end of the spectrum as a medium-term effort to eradicate heart disease. When I say medium term, I mean in our lifetime from the point of view of genetics.

I still believe heart disease is the largest cause of death worldwide. How exciting it is to be on the cusp of the forefront of the leading edge of research that hopefully will eliminate, from a genetic point of view, heart disease in mankind. I mean, how noble is that?

The Beat: How do you feel when someone from the Heart Institute comes to tell you about their latest genetic discovery?

John Ruddy: They do that all the time!

The Beat: What does that mean to you? Is it exciting?

John Ruddy: You know I think, first of all, I'm always excited to hear that they're making progress in their work. As a layman, it's difficult for me, sometimes, to appreciate how significant that particular discovery may be or what its applications may be, but I can tell you that I'm certainly impressed with their enthusiasm as they communicate it to me, so I presume there's something of value that will come from it.

But really, it comes back to the benefit to the overall community, not just the Genetics Centre but the benefits accrued at all the levels. It has enhanced health for our local community, created employment and research activities, and I think that just builds a broader base in terms of diversifying our global economy. And I think that's a good thing.

The Beat: Do you have kids in your family?

John Ruddy: I have two teenage daughters, one is 19 and the other will be 17.

The Beat: So, what's it like to look at them knowing there's a real chance of ending heart disease?

John Ruddy: It's very positive. They could face a lot of other challenges, but you know what? This will be something that they will benefit from, that the community at large will benefit from. It's very encouraging.

The Beat: Clearly, you're a compassionate individual. So, why didn't you become a doctor?

John Ruddy: (laughing) Oh, it's too difficult and lengthy an educational pursuit. I'd have never finished!

Where We Go from Here

While cardiovascular genetics is a relatively young area of research, the Heart Institute has made remarkable progress in identifying genes associated with disease. The ultimate goal, though, is to put the rapidly accumulating genetic information to work in the clinical setting and, eventually, eliminate heart disease by personalizing treatment and prevention.

Genetics is already impacting cardiac patient care. A patient's genetic profile can help guide drug selection and dosage for statins and some blood thinners. In the case of inherited arrhythmias, genetic screening provides invaluable information about the risk borne by patients and family members for life-threatening conditions.

Yet, the application of genetic information to patient care is largely uncharted territory. This spring marked the publication of the very first case study in which a patient was assessed for cardiovascular disease using his fully sequenced genome. How best to integrate this kind of information in individual prevention and treatment is something medicine is just beginning to address.

A key next step in cardiovascular genetics will be to use this expanding knowledge base to develop new drug treatment options. For this to happen, we need to continue to identify genes responsible for heart disease. But, as well, we need to begin to pin down how known genes function. What are the mechanisms by which they increase risk or protect against disease? What are the proteins or signalling pathways involved? Characterizing these mechanisms will identify the targets and linkage points where new therapies can intervene and alter the progression of heart disease.

To this end, the Heart Institute plans to open a Cardiovascular Gene Function Centre to complement the gene discovery effort ongoing in the Ruddy Canadian Cardiovascular Genetics Centre. The planned 3,100-square-foot Centre would be created from an amalgam of laboratory renovations and equipment purchases.

While about half of a person's susceptibility to heart disease is genetic, we can't ignore the other 50 per cent—namely, environment and lifestyle—if we hope eventually to make heart disease a thing of the past. The rapid progress being made in diagnostic and preclinical testing will strongly enable early detection and prevention in the coming years. The ultimate strategy will be personalized medicine, whereby prevention and treatment are customized to the individual's genetic variants.

The Heart Institute also plans to develop a Centre for Wellness and Prevention that will provide comprehensive testing of risk factors predisposing individuals to heart disease. This will include conventional risk factors, such as blood pressure and cholesterol, and genetic risk factors, such as 9p21. This walk-in clinic will be open to the public and for regional referrals. Patients will be provided with individual risk profiles, including referral to a specialist if appropriate. Armed with information from this Centre, the public will be better positioned to make the necessary lifestyle changes to improve its heart health.

With this balanced approach, pursuing new knowledge and treatments, as well as effective prevention strategies, it is conceivable—and we feel it is highly likely—that heart disease can be eliminated in Canada in the coming decades.

MORE INFORMATION ONLINE

Read the Heart Institute's Strategic Plan:

www.ottawaheart.ca/about_us/our_strategic_plan.htm