

# World's first gene

## and how it turned Ottawa into a leading

When asked how and why he embarked on what turned out to be one of the most significant medical research projects ever, Dr. Ronald Worton answers with one word: serendipity. Then he starts drawing the X chromosomes of a young girl from Belgium. The first time he saw those chromosomes was in 1977, but he still remembers exactly how they looked and the precise pattern of light and dark bands.

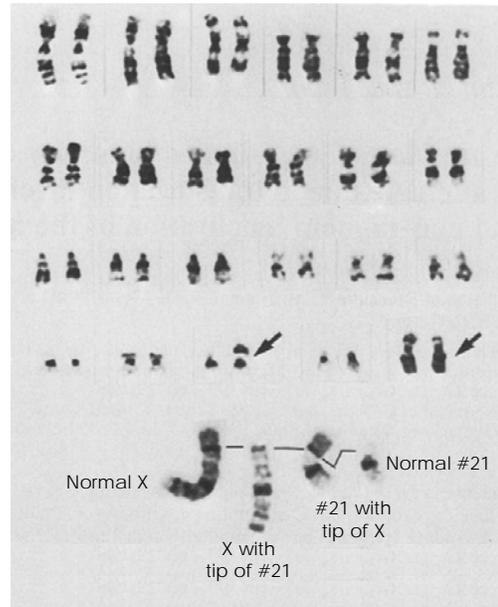
Eventually, these chromosomes would lead to the discovery of the gene responsible for Duchenne muscular dystrophy (DMD), the first disease-causing gene ever to be identified without already knowing the protein encoded by the gene. It would also turn out to be the largest human gene ever discovered, more than 100 times the average size. The knowledge gained along the way opened the gates for a flood of gene discoveries in the late 1980s and early 90s, and paved the way for the Human Genome Project itself.

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Dr. Ronald Worton

Although he made the discovery in Toronto, Dr. Worton has been a health research leader in Ottawa for the last 11 years, as CEO and Scientific Director of the Ottawa Health Research Institute, the research arm of The Ottawa Hospital and an affiliated institute of the University of Ottawa. By recruiting and supporting top



Top four rows show the chromosomes (in pairs) of the girl in whom the Duchenne muscular dystrophy gene was discovered. Pairs are supposed to be identical, but in the case of this girl, the tip of one X (magnified in bottom row) was switched with the tip of a chromosome #21.

scientists from across Canada and around the world, he has helped turn Ottawa into a major centre for neuromuscular disease research and stem cell research (the field that many believe will finally enable treatment of these diseases).

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The story of the discovery of the DMD gene includes many twists and turns. One of these is the fact that Dr. Worton's team identified the gene in a girl, while DMD almost always affects boys. The reason for this has to do with our chromosomes – the structures that contain all our genes, arranged in a defined order. Humans have two copies of each chromosome, except for the "X" which is present in only one copy in males. DMD, like a number of other genetic disorders, primarily affects males because it is caused by a problem on the X chro-

# ry behind the genome project city for muscle and stem cell research

mosome. With only one copy of the X, males cannot compensate for these problems the way females can.

In the 1970s and 80s, Dr. Worton led the cellular genetics lab at the Hospital for Sick Children in Toronto and he was particularly interested in disorders involving chromosomes. In 1977, a pediatrician from Belgium named Dr. Christine Verellen joined Dr. Worton's research group, and she brought with her the famous chromosomes that would lead to the identification of the DMD gene.

"In Belgium, Dr. Verellen had treated a young girl with DMD, and when we looked at that girl's chromosomes, we could tell immediately that something was wrong," said Dr. Worton. "Every chromosome has a unique pattern of light and dark bands, but in this girl, the patterns on some of the chromosomes were different. It looked like the top end of one X chromosome had been exchanged with the top end of chromosome 21 – a rearrangement called a 'translocation'."

They published this finding and by 1982, research groups around the world had identified five more cases of females with muscular dystrophy with similar X chromosome rearrangements.

"The simplest interpretation was that in each of these young girls, the gene for DMD was disrupted by the break in the X chromosome," said Dr. Worton. "There is a genetic reason why these girls showed symptoms of DMD, even though they still had one normal X chromosome, but the more important part of the story is that this obvious visible break suggested a method by which we, and others, could identify the gene."

Two main groups led the global race to identify the gene: Dr. Worton's team in Toronto and Dr. Lou Kunkel's team in Boston. They would take very different approaches and encounter unique difficulties. Dr. Worton's group purified chromosomes from the Belgian girl by fusing her cells with mouse cells, and then

using a special genetic trick to select only those cells with a single human chromosome – the one with the disrupted DMD gene. That turned out to be the easy part. It took nearly a year of tedious molecular work – doing experiments with exotic names like "zoo blotting" and "chromosome walking" to find the translocation breakpoint, subsequently confirmed to be within the DMD gene.

Although the Worton and Kunkel labs were in steep competition, they also worked together and shared results and they agreed to publish part of their work together in 1986. A media frenzy followed, but in yet another twist, it turns out that one of the most meaningful stories, which happened just weeks after the discovery, was never told.

"Dr. Margaret Thompson at SickKids was providing genetic counseling for a woman from a family with a history of DMD," said Dr. Worton. "Her brother and nephew were both affected, and she was a carrier. She desperately wanted another child, but she knew that any boy that she had would have a 50 per cent chance of developing the disease. When Dr. Thompson saw her she was already pregnant and we decided to offer prenatal diagnosis. We tested her brother and identified the mutation in the DMD gene. We then tested the woman, and confirmed that she carried a copy of the



Dr. Ronald Worton in his muscular dystrophy research laboratory in Toronto

defective gene. Finally, we tested cells from her unborn baby, and determined that it was male, but did not have the mutant gene. Four months later she had a perfectly healthy boy. She sent me pictures, and she was so thankful. This was the world's first pre-natal diagnosis for muscular dystrophy – a memorable highlight in my career."

Research over the next few years revealed that the DMD gene encodes a protein, named "dystrophin" by Dr. Kunkel, that helps strengthen muscle fibres and protect them from injury. Dystrophin was shown to be part of a multi-protein complex that resides at the muscle fibre membrane, and in people with DMD, this whole complex is disrupted. This leads to muscle weakness so severe that by age 9-12, most patients will be confined to a wheel chair. Research on dystrophin opened the door to further research on this multi-protein complex, with several of the proteins involved now shown to be defective in other forms of muscular dystrophy. In a broader context, the techniques learned in identifying the DMD gene led to a golden age of disease gene discovery, which included identification of the Cystic Fibrosis gene by Dr. Worton's colleague at SickKids, Dr. Lap-Chee Tsui.

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In May 2007, the 20th anniversary of the discovery of the DMD gene will be celebrated in Ottawa at an international symposium ([www.ohri.ca/dmdsymposium](http://www.ohri.ca/dmdsymposium)). Keynote speeches will be given by both Dr. Worton and Dr. Kunkel. DMD researchers from around the world will dis-

cuss the state of their research and how best to move forward.

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Although there is no cure yet, there has been great progress, especially in stem cell research. Just this year, a team from Italy showed that dogs with DMD could be treated quite effectively by a transplant of normal muscle stem cells. At the OHRI, Dr. Rashmi Kothary's group recently revealed a new approach to improve DMD gene therapy in mice, and Dr. Michael Rudnicki and Dr. Jeff Dilworth have made great strides in understanding how stem cells give rise to muscle tissue, and how best to harness this process for disease treatment. A number of researchers at the neighbouring CHEO Research Institute are also making important progress on related neuromuscular diseases – myotonic dystrophy and spinal muscular atrophy.

"I'm really looking forward to this meeting," said Dr. Worton. "Not only will it be an opportunity to see many of my old friends from around the world – many who played a significant role in the discovery of the DMD gene – but it will also be a poignant reminder of how a simple idea led to a whole new field of research, and to new approaches to therapy for this devastating disease."

## A personal reflection on 11 years at the OHRI

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fellows and a total staff of over 1,200 people who call the OHRI home. The research budget has increased from zero in 1986 to about \$17 million in 1996 to over \$75 million in 2006-07. Over the last six years we have had exemplary support from The Ottawa Hospital, the University of Ottawa and from the OHRI Board of Directors. In particular, I would like to acknowledge the phenomenal support of Hospital President, Dr. Jack Kitts, University President, Dr. Gilles Patry and Board Chair, Jacquelin Holzman.

Now we are about to begin the third decade with a solid base of outstanding researchers and a new leader, Dr. Duncan Stewart, a cardiologist and stem cell researcher. He will bring a new perspective – that of the physician-scientist – to the Institute and will undoubtedly bring a greater integration with the scientific staff at the University of Ottawa Heart Institute. He will lead the development of the next scientific and business plan of the Institute and will spearhead a proposal to the Research Hospital Fund of the Canada Foundation for Innovation for new facilities that will propel the Institute to a new level of excellence.

And what will I be doing? As Chair of Research Canada I will continue to advocate for stronger and better planned federal government support. As Vice-Chair of the Board of the Ontario Research Fund I will be working with the provincial government to maximize the opportunities for health research in the province. Thus, I am not leaving the field, but am only changing my role to a more global one; one that I hope will benefit the OHRI along with all health research institutes in Canada.