2016 William Allan Award: Human Disease Research: Genetic Cycling and Re-cycling[1]

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Thank you, David, for that very kind introduction. I do remember that early Apple computer game. We actually created a much better game by putting those mutations in. The other thing I remember is the lunchtimes we spent playing Middle Earth along with Joe Kernan at Pritchett, also an experience.

Human genetics is essentially a cooperative endeavor, and I am really humbled and proud to be here accepting the William Allan Award. But the work that I have done over the years has always been done with collaborators, so I would like to sincerely thank the many collaborators, trainees, and dedicated technical staff who all helped me to reach this point, as well as helped to forward the particular disease research that we were interested in. I'd also obviously like to thank those who nominated me and said such nice things, as well as the selection committee, which chose me for this award.

It is particularly apt that I formally receive the Allan Award here in Canada. I did start out at the University of Ottawa. Having grown up in Ottawa, I decided to go to school there. I was introduced to a typical university biology department and all kinds of diversity such as axolotls, halophilic bacteria, electric eels, and my personal favorite (because I spent a summer working on them in Donn Kushner's lab), blue-green algae in Ottawa River fish slime. But really what I was captured by at the University of Ottawa was the bilingual beauty and logic of molecular biology taught by Gordin Kaplan, an eminent Canadian scientist who actually originated in New York City but spent his academic career in Canada and taught in both French and English. He used to alternate lectures in the two national languages. With his encouragement, after graduating from the University of Ottawa, I moved for graduate school to the Department of Medical Biophysics at the University of Toronto, where the Ontario Cancer Institute represented a truly interdisciplinary research environment set in the context of a medical treatment facility, the Princess Margaret Cancer Hospital. It was really an amazing experience to be within that setting and see both the basic research that was being done and the goal for which it was being done, i.e., patients with the medical problems it was trying to solve.

However, on the day I arrived to do my graduate work, David Housman, my thesis advisor, announced that he was moving to the Massachusetts Institute of Technology (MIT), and I had to make a choice. I chose to go with him, so within a year we moved to MIT. It was a really exciting time because I was moving into the nascent, controversial field of recombinant DNA, which was just getting started at that point. It was also an interesting time to be a graduate student because very shortly after I arrived, fears of Frankenstein monsters and danger warnings caused the Cambridge City Council to place a moratorium on all recombinant DNA work within the city limits, prompting some at Harvard to move to other institutions in order to be able to do their work. In my case, as a graduate student, I didn't have that choice. I hung on, and a little while later the City Council relented a bit; they allowed mammalian DNA cloning but only under the very strict containment conditions of a P3 facility, where you had to gown up to do the work, and you had to use highly disabled vectors that were extremely difficult even to

[1]This article is based on the address given by the author at the meeting of the American Society of Human Genetics (ASHG) on October 19, 2016, in Vancouver, Canada. The audio of the original address can be found at the ASHG website.

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propagate. But it was an experience to spend time making packaging extracts in the cold room to generate your recombinant libraries and then having to gown up, go into a negative-pressure room, and work in a laminar flow hood before you were actually allowed to use them.

Well, I finally did graduate, but I actually didn't accomplish any of the gene-cloning goals that I had when I had begun the work at MIT. Because I had started out working on erythroleukemia cells, one of the globins was the obvious gene to clone, but these were quickly done elsewhere. So I moved on to a couple of other genes; at one point, I was even trying to clone the soybean globin, leghemoglobin, but plant DNA turned out to be really hard to work with. In the end, I managed to graduate by cloning DNA from somatic cell hybrids, a cell technology whose potential I had learned to appreciate from the work of Lou Siminovitch in Toronto and Bud Baker in the lab next door at MIT on Chinese hamster ovary (CHO) cell mutants and by collaboration with Ted Puck and his colleagues on CHO cells fused with human cells to segregate human chromosomes.[1] We found that we could make genomic libraries and specifically identify the human DNA, so if you could create a human-rodent hybrid cell with the particular human genomic region that you wanted in it, you could clone the DNA from that region.[2]

I graduated with my PhD in 1980, but a year after I had left Canada in 1975, Maria Frangione, also from Ottawa, and I had been married, so she had moved down to Boston to complete her medical training and pediatric residency. In 1980, we were faced with a decision—a tough decision that was molded by many factors that I won't go into. She decided that she would join a group medical practice in the Boston suburbs, and I, despite the career risk, skipped a planned postdoctoral training position at the California Institute of Technology and moved instead across the Charles River to Massachusetts General Hospital (MGH), where Joseph Martin, then the chief of neurology, encouraged me to start a lab to apply recombinant DNA techniques to the fascinating mystery of Huntington disease (HD). When I first started, I had salary coverage for about 3 months. Fortunately, the NIH Center grant that included my project was funded, although the part of the grant that was supposed to supply HD families was cut, so that created an issue. However, I still had a job, and I was able to undertake a new approach to this devastating inherited neurodegenerative disorder, which at the time was known for its autosomal-dominant inheritance pattern, its typically mid-life onset of a progressive, writhing, dance-like movement disorder, loss of neurons in the striatum of the brain, and a very protracted one- to two-decade decline to ultimate death, with no effective treatment.

The research approach on which I set out was ultimately conceived as a cycle (Figure 1). It is a cycle that has become increasingly powerful over the past four decades as technologies have rapidly improved. The strategy aimed first to apply genetics through molecular biological techniques to identify the causative gene by its chromosomal location without any prior knowledge of the nature of the genetic defect or the altered protein product. It was very new because at the time, if you wanted to clone a gene, you had to know the protein sequence. So, there was no direct approach to a gene such as the HD gene, because you didn't know what it was, and if you made a guess, you were likely to be wrong. But, we reasoned that we could find the chromosomal location. Then, once we knew that, we would clone the gene, we would figure out the mechanism by which the mutation worked to cause the disease, and ultimately we would use that information to deliver benefit back to the families in terms of better diagnostics, disease prevention and management, and a rational mechanism-based treatment. This "genetic research cycle" begins and ends with human patients, but it also emphasizes the interdisciplinary, cooperative nature of human genetic research in which we are all engaged, and if you think individually about what you are working on, it helps to position individual research efforts into a larger context.

In 1980, this unbiased genetic strategy began with applying anonymous DNA variations, detected as altered restriction enzyme cleavage patterns,[3] in order to track pieces of chromosomes through families and perform genetic linkage analysis. We were fortunate to be able to connect with Nancy Wexler. I pointed out that the part of the NIH grant that was supposed to supply families was cut. It turned out that Nancy was at the NIH, where she was our program officer for this grant. She got on a plane and went down to Venezuela, where she started to collect blood samples from this very large HD family that she knew about. She also connected me to Mike Conneally, who ran the HD Roster at Indiana University. The combination provided many, many families from all around the world, particularly in the United States. Nancy ultimately left the NIH and went on to Columbia University and to running the Hereditary Disease Foundation, which Milton Wexler, her father, had started. Mike spent his years at Indiana University continuing with HD.

The linkage effort led to the mapping of the HD genetic defect to chromosome 4.[4] It was the first time that an autosomal defect had been mapped with only DNA markers and no prior knowledge. I remember the day in 1983 that I read the gels and placed the genotypes on the pedigrees. You didn't need to calculate a LOD score. You could look at it and say, "That's linkage." I experienced for the first and strongest time an incomparable feeling: that of uncovering a definitive, scientific fact that no one had ever known before. That feeling is really indescribable.

This success came far earlier than anyone expected, and it set off a torrent of other DNA linkage studies in many laboratories, including my own. It created a debate about responsible presymptomatic testing in an untreatable disease with psychiatric features, and it acted as an early impetus to discussion of a human genome project.

In expanding the application of this strategy, my group helped to create the Molecular Neurogenetics Unit at

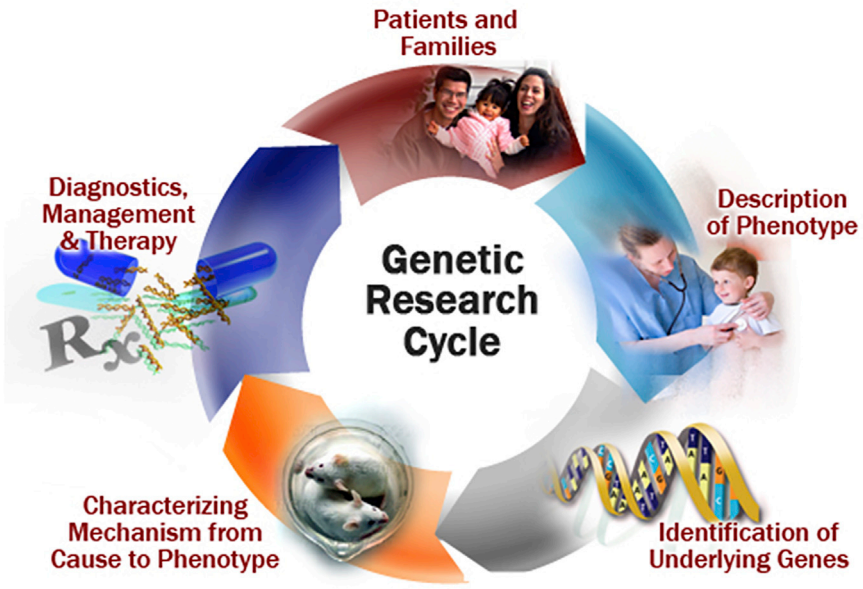

Figure 1. The Human Genetic Research Cycle

This cycle presents a research paradigm that is broadly applicable to all human disease. It begins with a "genetic discovery" phase, which combines phenotype descriptions in patients and families with genetic strategies (e.g., linkage, association, and structural analysis) to discover genetic factors that contribute to the phenotype, be they causative genes, risk factors, disease modifiers, determinants of response to treatment, etc. The discovery of contributing DNA variation then leads to a phase in which a variety of disciplines (molecular biology, biochemistry, physiology, protein chemistry, etc.) are applied in humans and in genotype-driven model systems (cultured cells, mouse models, lower organisms, cell-free systems, etc.) to define the mechanism by which the genetic variation affects phenotype. In humans, this phase continues to refine the relationship between genotype at the contributing variant(s) and phenotype, which could involve any of a wide variety of descriptors and/or readouts. These human genotype-phenotype relationships then permit the disease relevance of the mechanisms defined in model systems to be judged by whether they vary similarly with genotype. Because disease ultimately affects the entire organism (human or model), this phase can be thought of as "systems genetics," i.e., systems biology in which the perturbations to the system are genetic ones whose genotype-phenotype relationships are known in humans. The knowledge that emerges can then be applied to returning benefit in a "genetic medicine" phase (sometimes termed genomic medicine, although that term focuses more on the tools employed than on the fundamental nature of the critical information, which lies in genetic variation) through improved diagnosis, management, and prevention of disease and through the application of rational mechanism-based treatments. Multiple turns of the cycle can involve either different phenotypes in the disease or new phenotypes defined by response to the interventions dictated from the genetic medicine phase. The power of this cycle continues to grow in all of its phases. Genetic discovery is accelerated by the increasing size and number of population-based and disease-specific biobanks, the connection to electronic medical records, and increasingly sophisticated phenotyping methods, including advanced bioimaging, wearables, and "omics" technologies. Systems genetics benefits from all of these, as well as the availability of patient-derived induced pluripotent stem cells and their derivatives, genome-editing technologies for creation and manipulation of genotype-driven model systems, increasing sophistication of hypothesis-free computational modeling, and incorporation of gene-gene and gene-environment interactions. Genetic medicine can capitalize on all of the technologies and approaches mentioned to provide better, more specific diagnosis and monitoring of disease state to define the best potential sites and means of therapeutic intervention and to support genetic stratification for more effective clinical trials and treatments.

MGH, which has contributed over the years to chromosomal mapping of disease genes in a wide variety of human disorders, including, among others, neurofibromatosis 1, neurofibromatosis 2, von Hippel-Lindau disease, early-onset torsion dystonia, hyperkalemic periodic paralysis, familial Alzheimer disease, familial dysautonomia, familial amyotrophic lateral sclerosis, Batten disease, achondroplasia, mucolipidosis IV, and biotin-responsive basal ganglia disease.[5–17] At the time, although we were certainly interested in driving this technology, we had not given up on HD. We were only at the first part of the cycle, and we wanted to continue to drive the disorder around the cycle to achieve the original goal.

The next step was to move from the initial linkage marker, an anonymous piece of DNA known as *D4S10*, to the identification of the disease gene. We were faced with this task in 1983 at a time when technologies for high-throughput sequencing and long-distance physical mapping of DNA had yet to be developed. This quest was again pursued collaboratively, with my Massachusetts HD Center Without Walls colleagues Marcy MacDonald and Richard H. Myers, and through an international collaborative group that included Peter Harper and Russell Snell in Cardiff, John Wasmuth at the University of California at Irvine, Hans Lehrach in Germany, David Housman across the river at MIT, Francis Collins in Michigan, and many others. It took a decade. It was a decade that saw the introduction and application to this disease of many new technologies such as the polymerase chain reaction (PCR)—does anyone remember the days before PCR?—pulse-field gels, linking and jumping libraries, radiation hybrids, and yeast artificial chromosome cloning, but ultimately the guidance of genetic analysis produced the discovery of the HD gene.

After HD families had been contributed by many clinical and research collaborators around the world, we continued to pursue linkage analysis, now by incorporating multiallele VNTR (variable number of tandem repeat) markers and PCR-based simple sequence repeat (SSR) markers. This narrowed the location of the defect to the terminal cytogenetic band of the chromosome 4 short arm, but it narrowed it only to within 2 Mb given the very low recombination in this region. There were no recombination events in HD families to narrow the region further, so we turned

to examining the history of this chromosomal region in a very early application of mapping by genetic association. Although we began with individual marker association, we quickly gained an appreciation for the value of building haplotypes, particularly with the high resolving power of multi-allele markers.[18,19] That haplotype analysis implicated multiple origins of the HD mutation and narrowed the candidate region from 2 Mb down to about 150 kb.

While the MacDonald lab was evaluating a CAG repeat polymorphism as an additional PCR-based marker to add to the haplotype, it was found that the CAG repeat was expanded beyond its normal range of 6–34 repeats on all HD-associated chromosomes and was the cause of the disease.[20] I remember well the day that Marcy and her postdoc rushed into my office with the critical autorad still dripping from just being developed. It was another one of those incredibly satisfying scientific moments, this time tinged with considerable relief after 10 years of searching. The repeat proved to encode a polyglutamine tract near the amino terminus of a large anonymous protein, which we named huntingtin.

There were similar positional cloning efforts for many other disorders within the unit. Having achieved linkage, we attempted ourselves, or to help others, to move these disorders around the cycle. In almost all cases, when we found a disease gene, the gene was unexpected.[11,12,21–25] It was not something you would have predicted in advance and found by a candidate approach. The unbiased approach, therefore, opened entirely new areas of biology.

Among these, the familial dysautonomia gene, discovered with Susan Slaugenhaupt,[24] is an example of taking advantage of opportunity and jumping forward in the cycle to develop a therapy based upon the mutational mechanism, even before fully understanding the downstream consequences that produce the disease phenotype. As a potential treatment, Sue's lab is actively investigating a class of small molecules related to kinetin, which we found efficiently corrects the tissue-specific splicing defect that causes this disorder in vitro and in vivo.[26] So you don't necessarily need to understand pathogenesis in order to treat—you just need an entrée, and the unbiased genetic approach can get you to that entrée.

In another example of a genotype-driven approach, in relevant human cells you can jump directly to small-molecule screening for potential therapeutics if you use appropriate knowledge of the disease and the genotype. With Vijaya Ramesh and more recently as part of the Children's Tumor Foundation NF2 (neurofibromatosis 2) SYNODOS Consortium, we have relied upon isogenic wild-type and *NF2*-null human arachnoidal target cells to identify compounds of potential value for the treatment of meningiomas.[27,28] Early hits from this genotype-driven approach are already going to clinical trials for meningioma.

You can't jump forward in all disorders. You sometimes need to work on precisely what the disease gene is doing to cause the pathology. The basis for that is again genetics. Genotype-phenotype correlations can guide you around the cycle. Model systems are great, but the gold standard is the human disease. An animal model's relevance can be judged by how well it fits the genotype-phenotype relationships of the human disease. And when I say phenotype, I don't mean the end-stage phenotype or trying to reproduce every aspect of the human disorder. I mean the proximal effects of the mutation at the molecular and biochemical levels that initially trigger what, downstream in humans, ultimately becomes a severe whole-organism phenotype.

In HD, the human genotype-phenotype studies immediately revealed a very strong correlation between the length of the expanded CAG tract in the HD gene and the age at onset of diagnostic motor signs,[29–31] indicating by itself that the length of the mutation is the primary determinant of the rate of pathogenic process that leads to clinical onset. This fundamental relationship has formed the foundation of our attempts to relate the DNA mutation to the mechanism of pathogenesis, an effort that is being pursued with a team of collaborating investigators in the MGH Molecular Neurogenetics Unit: my close colleagues Marcy MacDonald, Vanessa Wheeler, Jong-Min Lee, Ihn Sik Seong, and Jim Walker. The accumulated evidence suggests that the HD CAG expansion confers huntingtin with a gain of function that acts through progressive enhancement or dysregulation rather than loss of normal activity. The parameters of this gain of function, including its being completely dominant,[32] have been defined by continued human genetic studies and are being investigated in various systems where potential consequences of the mutation can be judged for relevance to the disease process on the basis of their co-variation with the CAG repeat. That quantitative relationship gives you an entrée to judge differences as to whether they are meaningful or they are noise.

The ultimate goal in HD and these other disorders is to complete the cycle with a mechanism-based treatment. Whether aimed at the mechanism of mutation or the mechanism of pathogenesis doesn't matter, because what you really want is to deliver an effective therapy. The proof of principle that it is possible to reduce the rate of pathogenesis in HD prior to clinical onset has again come from human genetic studies. From the point of view of our research paradigm, we began to go around the cycle in HD a second time. We simply changed the disease-related phenotype that we were looking at. In this case, we started the genetic research cycle again by using information from large natural history studies in HD (including the COHORT[33] and PREDICT-HD[34] studies of the Huntington Study Group and the REGISTRY study[35] of the European Huntington's Disease Network) where the age at onset of the individuals had been recorded. After the effects of CAG length had been accounted for, variation in the age at onset remained, and we had built up evidence that it was familial, so we approached it with the same genome-wide association approach that you would use to look for risk genes in common disease. In this

case, we weren't looking for risk; we were looking for disease-modifying loci that either hasten or delay HD onset in a true genetic interaction. We did find them, quite strong ones in some cases.[36]

As part of the Genetic Modifiers of HD (GeM-HD) Consortium, which was funded by the CHDI Foundation, we have so far been able to look at more than 4,000 HD individuals. Data in the hopper right now will double that number for age at motor onset, but also, because the subjects come from these large natural history studies, we are able to look at a long list of other phenotypes. The important point here, however, is that what these modifiers are doing by changing the age at onset is to change the disease process before the clinical symptoms ever emerge. So they give hope that it is possible to delay the disease and maybe to prevent emergence of the disease at all if we can identify precisely which genes are being affected, how they work, and whether they and the processes they participate in can become targets for rational treatments, i.e., disease-modifying pharmacological interventions that, with yet greater strength, could replicate or antagonize the same biochemical effect as the genetic modifier. Such rational, mechanism-based treatments aimed at CAG-driven pathogenesis are expected to be most effective before to at least shortly after onset, since HD genetic studies have also revealed the surprising finding that the duration of actual clinical disease, from onset to death, is largely independent of the expanded CAG length.[37]

The same strategy of identifying disease-modifying genetic factors obviously applies to any other disease that we are trying to push around the cycle: the goal is to come up with rational targets. But technology has also moved on, and it is now possible to look beyond traditional small-molecule therapeutics. In fact, there is a large effort in HD, both in academia and in industry, to devise methods to suppress the expression of huntingtin, either overall or in an allele-specific manner. The earliest of these is actually in a human safety trial right now, but the advent of CRISPR-based gene editing has raised the further possibility of permanent, specific inactivation of the mutant gene. The proof of principle for this approach was recently reported by Jong-Min Lee's lab, which selectively deleted 44 kb of the HD gene (unfortunately renamed *HTT*), including the CAG repeat, by targeting Cas9 to protospacer adjacent motif (PAM) sites that are known to be polymorphic.[38] They are known to be polymorphic and on the HD haplotype because over the years, we have carried out very extensive haplotyping of the HD populations, and we know the order of frequency of diplotypes seen in Europeans and in some other populations.[39,40] You can choose PAM sites that are likely to be heterozygous in the individual by experimentally checking as few as five sites guaranteeing heterozygosity in 90% of HD individuals. The idea of editing the genome and targeting specific polymorphic sites is a powerful one if you have developed the background genetic information in the disorder. So I would urge anyone working on dominant disorders to understand the haplotypes behind their mutations and the diversity of those haplotypes.

The technological advances of the past 15 years have allowed movement around the cycle, now based upon genetic association in complex disease, but they have also allowed it with structural abnormalities, whereby you can begin the cycle with one individual rather than a population of several thousand for a common disease or with a family of 10 or 20 for rarer inherited disorders. Such an effort has actually been ongoing since the late 1990s, when we partnered with Cynthia Morton to build the Developmental Genome Anatomy Project. The goal of this project is to identify genes of importance in development by investigating balanced chromosome translocations associated with abnormal phenotype. We spent many years in the laborious effort of using Southern blots and moving along the chromosomes to try to clone breakpoints and identify disrupted genes. The rate of discovery increased dramatically when Mike Talkowski joined the lab as a postdoc to develop a technique using next-generation sequencing to identify these genes. In very short order, we went from doing one or two breakpoints per year to dozens per year so that now hundreds have been analyzed. We have had an impact on the diversity of genes implicated in neurodevelopmental disorders, and these discoveries have propelled Mike's lab (now independent) and my lab to become very interested in a genetics-driven approach to autism and abnormal development. Notably, we implicated disruption of chromatin and transcriptional regulators as a significant contributor to autism and found that many neurodevelopmental loci are associated with phenotypes across traditional diagnostic boundaries, including both childhood and adult disorders.[41,42] The same technology has also had an impact on prenatal diagnosis and the specificity of information that you can give to parents. When karyotypic analysis reveals a balanced chromosome abnormality, you can apply rapid sequencing to determine exactly what gene is being disrupted and what the potential effect is.[43,44]

The attractiveness of the genetic research cycle as an organizing principle led in 2003 to the formation of the Center for Human Genetic Research (CHGR) at MGH. Until April of this year, I had the privilege of directing this cross-departmental, interdisciplinary center in the setting of a major hospital—bringing me full circle from my early graduate student days to doing interdisciplinary research in a setting where the hope for patient benefit is felt. The CHGR has the goal of advancing knowledge and treatment of disease by applying human genetic strategies. It has grown to more than three dozen faculty with expertise in each of the three major interacting phases of the cycle: (1) genetic discovery, (2) genetics-driven analysis of overall disease mechanism, which I refer to as systems genetics, i.e., examining and understanding how the entire system changes when you change genotype, and (3) genetic medicine, which takes advantage of what you have discovered in many different ways not limited to the

general approach of small-molecule treatment of disease. Center investigators span pediatrics, medicine, neurology, psychiatry, and anesthesiology, among others. It is truly an interdisciplinary enterprise with different sets of both medical and research expertise. Because we were already very strong in Mendelian disease, much of my recruiting focused on common disease, and we were very successful in driving the cycle there as well. Many of our faculty, including Curt Stern Award winners David Altshuler and Mark Daly,[45,46] have made seminal contributions to the genetics of both common and rare disorders.

The technological and resource advances that continue to occur at an astonishing pace promise to make each of these phases increasingly powerful (see Figure 1 legend), suggesting that human genetics will fundamentally change the face of medicine in many, many ways not only for the disorders mentioned here but also in all aspects of health and disease. Ultimately, genetics is part of being human—part of how a human is going to express phenotype either in health or in disease. It is so integral that in my view, medicine in the future cannot be practiced without including a consideration of genetics.

Specifically applied to HD, the genetic research cycle has already had an impact on the patient population through better capacity for early or differential diagnosis and for prevention. It has revealed HD to be a lifelong disorder whose clinical manifestations emerge relatively late, leaving a wide window for therapeutic intervention to delay or prevent onset. The capacity that genetic analysis has provided to identify those who will show symptoms later in life has also energized clinical researchers to perform large natural history studies, which provide additional phenotypes to further accelerate turns of the cycle. Similarly, a worldwide community of HD researchers is engaged in trying to devise effective therapies either by targeting the HD gene itself or by developing drugs against other potential modifiers. The continuing advances in HD, building upon specific human genetic knowledge, offer the hope that the ultimate completion of the cycle through a rational treatment is no longer distant.

That is a day that I dearly hope to see before I retire.

I will close by saying thank you again to all those in the research and medical communities who have helped me personally and who have participated in the genetic studies I have mentioned, particularly my closest colleagues at MGH. I would also like to thank the diverse funders who have generously supported my work over the years.

I also want to thank my wife, Maria, for understanding, helping, and putting up with the demands of my research career for so many years and also my three sons—Matthew, Michael, and David—who helped me a lot when they were youngsters. They gave me innumerable hours of mind-clearing relaxation and inspiration while I watched (just to prove I remain a Canadian at heart) their many hockey games.

Most importantly, however, I want to thank the patients and family members who have willingly participated in research by donating their time and blood, and sometimes more, to the cause of discovering and alleviating the basis for their affliction while often knowing that it might help only the next generation and not themselves.

A sincere and heartfelt thank you to all.

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