equilibrium. In an age threatened by greenhouse gas emissions, these data should be of special interest. But the ecological damage documented in the New Zealand sites persisted even longer: It took several million years for New Zealand's flowering plants to regain the prominence they enjoyed in southern forests before the impact.

What is needed now is evidence of fern spikes on other southern continents at the time of impact. Fossil deposits may reveal just how the fauna of the Southern Hemisphere fared through these remarkable perils.

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PERSPECTIVES: GENOMICS

Genetic Association by Whole-Genome Analysis?

Pui-Yan Kwok

Geneticists have long dreamed of determining the genetic basis of disease susceptibility by comparing variations in the human genome sequences of a large number of individuals. But it has been considered an impossible dream because of the technical difficulties involved in obtaining human genome sequence data. For example, it took an international team consisting of hundreds of scientists many years just to produce a "working draft" DNA sequence of a reference human genome (*1, 2*). Furthermore, humans are diploid organisms containing two genomes in each nucleated cell, making it very hard to determine the DNA sequence of the haploid genome. Yet, armed with the complete DNA sequence of one of our smallest chromosomes, human chromosome 21, scientists at Perlegen Sciences (a subsidiary of Affymetrix Inc.) have undertaken a pilot study to demonstrate that this dream is within reach. On page 1719 of this issue, Patil *et al.* (*3*) report their scan of some 21.7 million base pairs of unique (nonrepetitive) DNA sequence in human chromosome 21.

These investigators set out to identify all sequence variations—called single nucleotide polymorphisms (SNPs)—in human chromosome 21 and to group them into blocks called haplotypes. First, they established human-rodent hybrid cell lines, each containing one copy of human chromosome 21 from a different individual. Then they performed long range–polymerase chain reaction (LR-PCR) to amplify the regions containing unique DNA sequences. Finally, they obtained the complete DNA sequences of all of the copies of human chromosome 21 using high-density oligonucleotide arrays. The Perlegen team scanned 20 different copies of human chromosome 21 across the unique two-thirds of the chromosome, and an additional 19 copies of human chromosome 21 across one-eighth of the unique DNA sequence. The authors conclude that just three common haplotypes can describe variations among 80% of the human population, a far smaller haplotype number than previously thought.

Although only 65% of all the bases on the microarrays yielded high-quality data, the 20 sets of data each containing 14 million base pairs still constitute one of the largest sequence comparison studies ever. For the most part, the results agree with other large-scale sequence comparisons in terms of the rate of discovery of SNPs, the unpredictable pattern of haplotype structure across the chromosome, and the lack of haplotype diversity along much of the chromosome. The haplotype patterns observed across such a large span of DNA are certainly interesting, but broad conclusions about haplotype structures within the human genome cannot be made with a high degree of certainty because the number of chromosomes analyzed is still quite small.

Although human geneticists and population geneticists will continue to debate the merits of the conclusions of this study, all will agree that it marks a dramatic shift in strategic thinking. Traditionally, one looked at a limited set of markers (even at 1 million SNPs, one is still looking at just 0.03% of the human genome) in a genetic association study where the genetic makeup of individuals with a disease is compared with that of healthy individuals. Now, one can aspire to analyze all of the unique DNA sequences in the genome simultaneously.

To ensure technical success, Patil *et al.* wisely adopted the best features of several previous approaches. First, they realized that haplotype information had been helpful in genetic association studies, and so they decided to physically separate the two homologous copies of human chromosome 21 up front. Although this was quite laborious, the resulting DNA samples yielded not only haplotype data but also data that were much easier to analyze on high-density oligonucleotide microarrays. Second, they avoided amplification of background rodent DNA (from the somatic cell hybrids) by designing LR-PCR assays with uniquely human PCR primers. If they had amplified human DNA sequences in short PCR assays, some amplification of the rodent background would have been unavoidable. Third, high-density oligonucleotide microarrays are most effective when the experiment requires only a small number of reactions (exemplified by gene expression studies where one RNA preparation is used to study the global expression pattern of a particular cell type). The authors followed the lead of previous groups (*4, 5*) and used LR-PCR products as the DNA targets in their experiments. This made it possible to have ~400 LR-PCR products for each hybridization experiment and thus to interrogate roughly 4 million bases simultaneously.

Patil *et al.* emphasize the "common haplotype structure" of the human genome. Their whole-genome scanning approach has defined and produced a dense set of SNPs that then have been used to select the most common haplotypes of the human population. Instead of endorsing this strategy, I suggest that we adopt the new thinking provoked by this study and work toward comparing whole human genomes when performing genetic association studies. To achieve this, a number of improvements will be needed. We must be able to convert diploid cells to haploid cell lines readily so that even very large population studies are possible. Likewise, the generation of DNA targets needs to be accomplished with much less effort. Patil and colleagues performed 3253 LR-PCR assays to scan the unique sequence of 1% of the human genome. Clearly, performing 325,300 LR-PCR assays is not practical. Perhaps a whole-genome amplification strategy would solve the problem. Resequencing by hybridization has obvious limitations. For example, duplicated sequence motifs cannot be analyzed by hybridization on microarrays. Similarly, certain sequence contexts will always yield low-quality signals. Some of the low-quality data points could

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be salvaged in association studies where the focus is not so much on the correct call of a particular base in the sequence, but instead on pattern alterations when the hybridization signals from two genomes are compared. The pattern changes could then be followed up using other types of analyses. Finally, it goes without saying that analytical tools and algorithms capable of analyzing data generated from whole genomes must be developed to handle the comparisons. It is no easy task to compare two genomes from each of 1000 patients with a particular disease against those from 1000 normal controls in order to identify the genetic factors associated with a disorder.

Despite the obstacles, Patil *et al.* have shown us that when one sets out to achieve the almost impossible and does something about it, we are one step closer to realizing the dream.

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RETROSPECTIVE

Jeffrey Isner, 1947–2001

Judah Folkman

A pioneer in cardiovascular research and gene therapy, Jeffrey M. Isner died at age 53 of a cardiac arrest on 31 October 2001. His loss is a terrible blow to the world of medicine, and particularly to the field of cardiovascular biology. A professor of Medicine and Pathology at Boston's Tufts University School of Medicine, he also held the positions of chief of Cardiovascular Research, chief of Vascular Medicine, and director of the Human Gene Therapy Laboratory at St. Elizabeth's Medical Center in Boston.

Isner's vision was to revascularize the blood-deprived (ischemic) heart using gene therapy to promote the sprouting of new blood vessels, a strategy called therapeutic angiogenesis. He began to treat ischemic limbs as a bridge to eventually treating the heart. In December 1994, after years of laboratory studies, he and his team at St. Elizabeth's performed the first human cardiovascular gene transfer for a patient suffering from peripheral vascular disease in the legs. Initially, patients received an intra-arterial injection of naked DNA encoding the angiogenic protein vascular endothelial growth factor (VEGF). Subsequently, patients received intramuscular injections of the DNA for VEGF, and many were spared a leg amputation. By 1999, Isner's team was able to report that injecting ischemic heart muscle (myocardium) of patients with naked DNA improved collateral blood flow to the heart. The VEGF gene was delivered by a catheter that reached the heart's left ventricle through a percutaneous insertion into the femoral artery. The tip of the catheter entered the left ventricle, and the DNA was injected into ischemic sites in the wall of the heart, as determined by an elegant method of electromechanical mapping. By August 2000, Isner reported that in 9 of 13 patients with advanced heart disease, inactive heart muscle was working normally. Recently, his team had submitted for publication a paper reporting the beneficial results of a placebo-controlled double-blind trial of VEGF gene therapy for ischemic myocardium. This week, investigators in Switzerland also reported a successful placebo-controlled clinical trial of angiogenic gene therapy of the heart, using a different gene but confirming the principle that Isner had pioneered (*1*).

In 1997, Isner's laboratory reported the surprising discovery that endothelial progenitor cells enter the circulation from the bone marrow and that their numbers can be boosted by systemic administration of VEGF. When Isner first presented this result at a Gordon Research Conference in 1995, it was met with disbelief. But Isner persevered because he could foresee the possibility of isolating these cells from a patient's blood and returning them to the myocardium to augment revascularization. What no one could foresee was how rapidly this discovery would be confirmed by other investigators—within a year—and how important it would turn out to be for tumor angiogenesis (the growth of new blood vessels that nourish tumors). This month's *Nature Medicine* carries a landmark paper detailing how circulating endothelial progenitor cells contribute to tumor angiogenesis (*2*).

CREDIT: ST. ELIZABETH'S MEDICAL CENTER

I knew Jeffrey as a colleague and friend, and had the greatest respect and admiration for his pioneering research. His patients were devoted to him. He was always in good humor. He made them laugh. He gave hope to the hopeless. Even if a leg had to be amputated, the patient felt deeply that everything possible had been done to avert this course. Isner was devastated by a temporary Food and Drug Administration (FDA) hold on his clinical trials, not for personal reasons, but because he worried about sick patients denied therapy. He was elated when the FDA subsequently gave him approval to move ahead. He was a wonderful mentor to his staff, students, and postdoctoral fellows. He cared for them as though they were family. He always gave them credit and worked hard to develop their scientific self-confidence. He was especially proud that of the 24 postdoctoral fellows that he trained, 16 had either won Young Investigator Awards from the American Heart Association, or were selected as finalists. When Takayuki Asahara's paper on endothelial progenitor cells was accepted by *Science* (*3*), he was invited by Isner to celebrate at one of Boston's finest restaurants. Isner's work has received worldwide recognition and numerous awards, including a 10-year MERIT Award from the National Institutes of Health, and in September of this year a 5-year Program Project grant for a Center of Excellence in Gene Therapy.

Jeffrey Isner left a path for others to follow. His legacy will continue as physicians learn to treat ischemia of the heart as a chronic manageable disease. The clarity of his vision is best evoked in a recent essay by Lance Morrow (*4*), who described the similarities of his own heart attacks and their therapy to those of U.S. Vice President Cheney. Morrow wrote, "A couple of years ago, I drew ahead of Cheney in the fancy-therapy category by having DNA injected into my myocardium in order to induce the growth of new vessels—angiogenesis, a still experimental but highly promising technique which has, in my case, worked miraculously well."

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