A photograph of Louis Kunkel, a middle-aged man with a receding hairline, wearing a green sweater over a light blue collared shirt. He is looking directly at the camera with a slight smile. The background is a laboratory setting with various pieces of equipment, including a metal stand and a control panel with dials. The foreground is heavily blurred, showing what appears to be a computer monitor and other lab equipment.

Louis Kunkel in
his laboratory at
Children's Hospital

The Gene Hunter

Louis Kunkel's 30-year quest to diagnose
and cure muscular dystrophy

by Victor K. McElheny

LOUIS M. KUNKEL is a wiry man of medium height with a lot of energy, quiet passion, and notable persistence. The professor of pediatrics and of genetics has very little interest in publicity, but nevertheless became a hero in the 1980s when he discovered not only a marker for the Duchenne muscular dystrophy (DMD) gene, but the gene itself and the protein it specifies, thereby facilitating the diagnosis of carriers and afflicted fetuses. Working at the nexus of lab research and clinical application during his 30 years at Children's Hospital Boston, he is still in touch with DMD patients and their families whom he saw long ago. Their suffering has impelled him to push the use of genetics and genomics in his quest to devise improved methods of diagnosis and treatment. His story is a sobering tale of how high the barriers still are for scientists striving to put genomics to work to attack a genetic disease.

DMD, the most common and severe of the two-score types of muscular dystrophy, condemns its victims—almost all boys—to a succession of surgeries, as well as a course of steroids that can do nothing more than delay their inevitable confinement to a wheelchair around the age of 10. They die in their twenties or thirties when their heart and respiratory muscles fail. A third of the victims also experience mental retardation. An added tragedy is that the disease emerges only gradually, becoming evident at the age of four or five when a child has trouble getting up or running. As Kunkel quietly told a group of visiting journalists in 1998, "Most Duchenne families are desperate. It's pretty sad to interact with them. I saw a boy at five. He's now 19, in a wheelchair, and needs a respirator. I took part in a benefit for his family. It's unusual for a basic research laboratory to interact with patients. It puts everything in perspective. It adds a dimension other than intellectual curiosity."

Despite that passion, Kunkel's search for a cure for DMD underlines how challenging the grand promise of genomics—to lay bare the causes of disease, and discover remedies—remains 10 years after the first human genome sequences were drafted. His work also illustrates the determination of a rapidly growing number of researchers around the world to intensify, not slacken, the search for genome-based therapy and prevention. Biologists working with those data seek the molecular causes, not just the symptoms, of diseases rare and common, in order to produce specific methods for combating individuals' illnesses.

But despite huge advances in understanding the functions of many of the 20,000 human genes, researchers are finding that the underlying genetic causes of a single disease may lie not only in simple substitutions of DNA subunits, but also in much larger deletions, insertions, reversals, and variations in the number of copies of repeated sequences. They are learning that diverse biochemical pathways in the living cell can lead to the same result. They confront the paradox that the multiplicity of causes may complicate understanding of a disease—and yet may open up more opportunities to control or prevent it.

The bewildering array of genetic abnormalities that can impair a biological system means that understanding disease from a genetic perspective will require scientists to sequence the genomes of vast numbers of individual patients—and then aggregate, store, and link such data to individual medical histories on a massive scale. As Kunkel and many others have realized, this re-

search paradigm raises competing ethical concerns over privacy and patients' rights to know whether they harbor a predisposition to an illness. The moment to resolve these big issues is nearer at hand than most people know.

WHEN KUNKEL BEGAN hunting for disease-related genes in the early 1980s, he focused on one of the several thousand rare diseases linked to mutations in single genes, not common diseases like cancer or diabetes. But the conviction among scientists and the public alike that genetics accounts for a large share of human disease risk was already common. Prenatal testing, starting with such diseases as Down syndrome and Tay-Sachs, began spreading in the 1970s, and intensified in the 1980s after the isolation of genes linked to Huntington's chorea, cystic fibrosis, breast cancer, and hundreds of other afflictions, including Kunkel's target: DMD. All this was happening barely a decade after biological science had learned to isolate genes, to transfer them from one organism to another for research and industrial purposes, and to spell out the subunits of the code of life embedded in DNA. Speaking to a group of reporters in 1989, Kunkel said that medicine had to that point focused on diseases caused by factors invading from outside, and was only beginning to understand hereditary diseases and cancers that arise within us.

Kunkel, in fact, is one of the researchers whose success in finding disease-related genes helped crystallize the idea of a Human Genome Project, the international drive to spell out all the subunits of human DNA. This largest of focused efforts in the history of biology has indeed produced an intellectual explosion full of surprises, such as a myriad of newly discovered genetic controls in the genome itself and the proteins that wrap around it. Accompanying technological advances are already enabling researchers to sample the genetic endowment of thousands of people and to read completely the DNA of hundreds of individuals, not only to pin down more causes of disease but also to begin guiding therapeutic decisions in the clinic. Last October, *Nature* published a survey of 93 major genome centers around the world that are using, in all, some 1,250 of a new generation of ultrafast sequencing machines. The journal estimated that 2,700 human sequences would be complete by the end of that month—and 30,000 by the end of 2011. Nonetheless, there is much impatience with the painfully slow emergence of genomics-based medical applications, resulting in criticisms that the whole enterprise has been hyped.

IN 1986, after Kunkel and three of his Children's colleagues cloned the gene for DMD, they immediately pressed forward on a year-long hunt for the gene's product, a protein that they named dystrophin. The mutation leading to DMD prevents the manufacture of this molecule—a crucial member of a complex of molecules that repairs muscles after the stress of frequent contractions. Without dystrophin, muscles tear and wear out prematurely as they flex, and can't be regenerated, leaving DMD patients with virtually no muscle at the end of their short lives.

As the Kunkel group discovered, dystrophin—like all the body's tens of thousands of proteins, including those involved in hundreds of genetic disorders—is made up of its own unique combination of the 20 types of small molecules called amino acids.

These amino acids are arranged in order according to the genetic code of DNA, itself spelled out by the four "bases" called ad-

enine, thymine, guanine, and cytosine (A, T, G, and C) that are strung along at right angles to the twin sugar-phosphate strands of the DNA double helix. Triplets composed of these four individual bases form code words, or “codons,” each signifying that a particular amino acid is to be installed at that point in the chain-like protein. There also are codons for starting and stopping, like the capital letter at the beginning of a sentence or a period at the end. The string of DNA codons spelling out a particular protein is copied into a “messenger” (made of the related chemical RNA) that moves out of a cell’s nucleus to the globular protein-assembly platforms called ribosomes.

Dystrophin, composed of some 3,800 amino acids, is a cruelly easy target for genetic mutations. Its DNA sequence is encoded by 79 separate stretches—called exons—that are scattered along two and a half million of DNA’s three billion “letters.” In DMD patients, a mutation adding or subtracting just *one* DNA letter shifts the “reading frame” so that the rest of the message becomes gibberish.

Because dystrophin is such a barn-door of a genetic target, new kinds of mutations keep springing up. In the 1980s, one-third of the 600 or so boys born with DMD in the United States each year were victims of a “sporadic” genetic change arising in their mothers. In the remaining cases, the mother had inherited the defect from *her* mother. An estimated one woman in 5,000 is a carrier, but many still do not know that. Parents-to-be often lack a family medical history that might alert them to have themselves or a fetus tested. By the time a boy is diagnosed with DMD, the physician’s sad duty is to tell the parents about the expected course of an illness with no cure. (Kunkel recalls one mother bringing her affected four-year-old to the clinic at Children’s, with a younger, as-yet-undiagnosed son in tow.)

Kunkel and his colleagues’ search for a cure, in rivalry and cooperation with scientific groups across the world, focused on finding a way to supply the missing protein. But how? Injecting the normal form can’t work because dystrophin is so huge it cannot penetrate the walls



Below: Difficulty getting up from the floor is one of the early signs that a boy has DMD. Weakened quadriceps force the child to first raise his posterior from a position on his hands and knees, and then walk the hands up the legs to raise his upper body, in a series of steps called Gowers’ maneuver. Above: By age 15, due to scoliosis of the spine and contractures of the hips, knees, and ankles that freeze the joints, the boy is wheelchair-bound.

of muscle cells. Several forms of cell-transfer or gene therapy have also been tried; all have failed. As with many rare but catastrophic inherited diseases, the quest has been urgent, but has proven long and frustrating.

In the early 1980s, Kunkel’s group had confronted almost-universal predictions that it would be impossible to map the X chromosome, already known to be the site of the genetic defect implicated in DMD. The old-fashioned molecular techniques for gene mapping were cumbersome and lengthy. DNA extracted from cells was handled and measured directly, and all the data were punched into computers by hand. To locate the specific site (which turned out to be on the short arm of the chromosome), they began “walking” in both directions along the strands of DNA, constantly comparing each stretch to the corresponding area of normal DNA to find missing sections.

After three years, the first fruits were “markers” near the culprit sequence; three years later, the gene itself was found.

In the past 30 years, Kunkel’s ways of teasing out genetic contributions to disease have changed dramatically, from a “wet” world of handling DNA samples to a largely “dry” one of automated instruments, computers, and elaborate software. These technological advances, certainly, have helped bring the finish line in the race for solutions closer. But the exponentially increasing volume of electronic data is creating huge challenges in interpretation.

Kunkel admits that he never expected it to take so long for therapies to emerge. Yet he and many others in academia and the pharmaceutical industry keep at it. Today, they focus much attention on the very genetic machinery that has gone wrong. Using a form of gene therapy that Kunkel calls “gene correction,” they hope to trick the protein-synthesizing machinery of muscle cells into creating at least a truncated form of dystrophin. In one current approach, researchers add special chemicals as an enzyme copies the DNA into messenger RNA instructions for making the pro-



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Even though progress in finding a *treatment* for DMD has been slow, one area has benefited enormously: the process of locating and sequencing the genes and their proteins has had a swift, dramatic, and increasing effect on the quality of *diagnosis*.

tein; that induces the copying enzyme to skip past enough of dystrophin's 79 exons to restore the correct reading frame of the rest. The resulting shortened dystrophin, ideally, would modify the severity of the patient's disease from the Duchenne variant to another, called Becker, that allows a longer and less painful life. (Becker victims are born with shortened dystrophin proteins.)

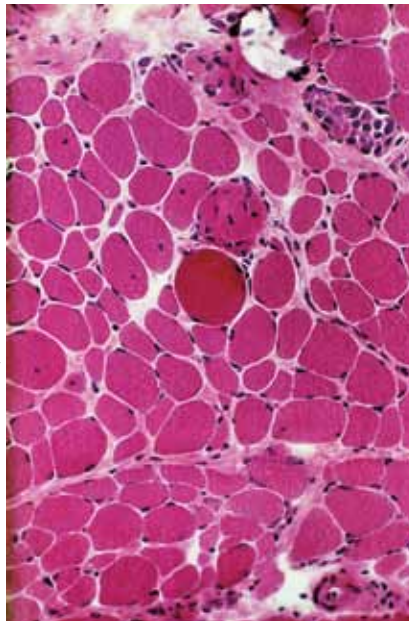
Other approaches judged promising are the use of chemicals to force the DNA to "read through" a premature stop codon, so that it makes a more complete protein, and an effort to boost the body's natural production of a related protein, utrophin, that might do at least some of dystrophin's work. Several of these methods have entered clinical trials in the United States and elsewhere.

Recently, Kunkel's lab has focused on another important front in seeking cures or palliation for DMD. This involves expanding the number of suitable laboratory "model organisms"—in which possible muscular dystrophy treatments can be tested—beyond genetically engineered mice. He and colleagues, including Jeffrey R. Guyon and Genri Kawahara, were excited to find that a form of muscular dystrophy occurs in one of the most-used organisms in developmental biology: the prolific, short-lived zebrafish, whose transparent embryos offer clear views of the effects of mutations on muscle. Seeking to reverse the zebrafish disorder, Kunkel's lab is testing a library of some 4,000 closely studied but outmoded or shelved drugs and has already found several that look promising.

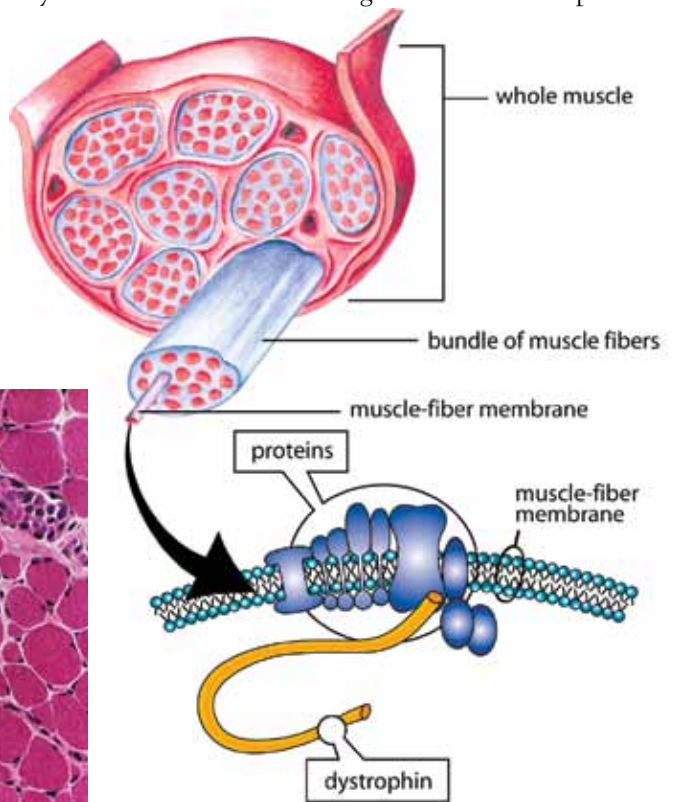
Meanwhile, even though progress in finding a *treatment* for DMD has been slow, one area has benefited enormously: the process of locating and sequencing the genes and their proteins has had a swift, dramatic, and increasing effect on the quality of *diagnosis*.

In medicine, where diagnosis tends to come before a cure, identifying a gene and its protein opens hitherto unavailable options. A woman who is a carrier of DMD, Kunkel points out, may

Right: Dystrophin is one of a group of proteins that surround muscle fibers and keep them working properly. The protein's absence in DMD patients leads to loss of muscle function. Below: A light micrograph of a section through muscle tissue affected by DMD shows wasting where healthy fibers (bright pink) have been replaced by fibrous tissue (light pink).



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have seen her brother die of the disease. She can now have an in utero test and, if the male fetus is normal, carry it to term. In-vitro fertilization, including genetic diagnosis before an egg is implanted, is increasingly available. Genetic understanding has also made diagnosis less painful and risky: muscle biopsies of newborns and young children can be replaced by simple DNA studies. This progress has led to a sharp rise in the proportion of American women who know they are carriers of the critical defect in DMD.

As a consequence, the frequent "sporadic," *de novo* mutations involved in DMD now account for more than half the live-births of victims, up from one-third a quarter-century ago.

Genetic diagnosis has been driven by new tools for exploring the body's hereditary endowment. One of these is the "DNA chip" (a small glass rectangle with a vast array of microscopic wells—a "microarray"—containing DNA samples that can survey variations in an individual's genome at a million points for

a cost of a few hundred dollars). Another is the DNA sequencing machine. The attendant computers and software have been increasing in speed and precision at a dizzying rate, rapidly reducing the cost of making a complete sequence of a person's genome. Less than a decade ago it was on the order of \$100 million or more. (The first complete sequence of a named individual, DNA co-discoverer James Watson, cost Connecticut-based 454 Life Sciences and its partner, the genome center at Baylor College of Medicine, \$1 million or so in 2007.) At a pace even faster than that in electronics, several generations of competing new technologies have cut the original price by four orders of magnitude to \$10,000, with a further cut to \$1,000 expected within a couple of years. (In 2010, San Diego-based Illumina cut its charge of \$48,000

down to \$19,500 for an individual, \$13,500 per person for a group of five, and \$9,500 when prescribed by a physician.) Several academic centers have also begun using a shortcut: sequencing only the “exome” DNA that codes for proteins—at a cost of about half that of a complete sequence—to find the exact genetic change causing an illness. Soon the cost of sequencing a person’s entire genome will likely equal the price now paid to test for a single genetic defect, promising further wholesale advances in the scale of genomic analysis.

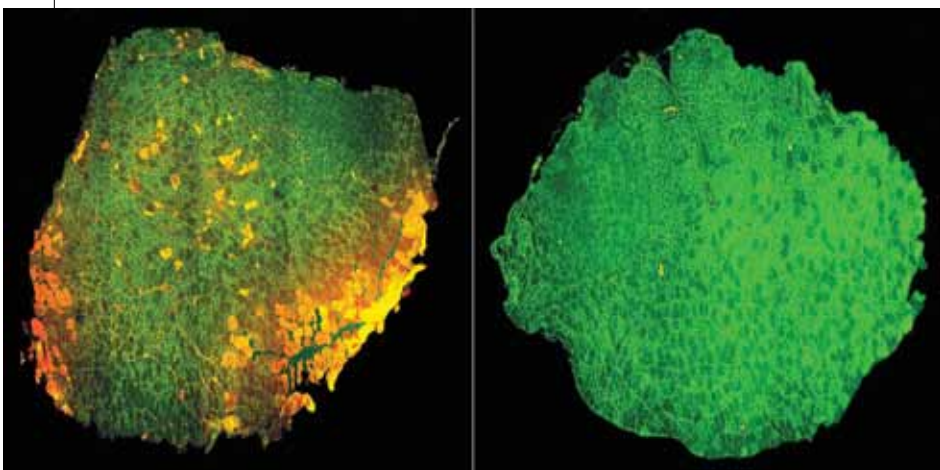
THESE RAPID DEVELOPMENTS have convinced Isaac S. (Zak) Kohane—a colleague of Kunkel’s at Children’s and director of the Countway Library at Harvard Medical School—and their co-workers that medicine is entering a world in which tens of thousands, even millions, of patients are likely to become participants in long-term genetic research. This trend intensifies concerns that first troubled Kohane in the 1980s, when he was simultaneously pursuing an M.D. at Boston University and working toward a doctorate at MIT in artificial intelligence as it related to medical decision-making. He became convinced that a patient’s medical data from all care providers must be not only centralized and easily available to the patient, but also easily accessible for research

participant model. Now Children’s has created the Gene Partnership, in which participants will be able to see their results if they wish. The program began enrolling participants last spring, starting with the hospital’s own developmental medicine department, and by late fall had recruited some 650 volunteers. The aspiration, Kunkel says, is to sign up a total of 10,000 within “a year or two.”

A primary goal of the Gene Partnership is to zero in on the actual mutations that contribute to disease. But to muster the statistical power necessary to detect these needles in a haystack, Kunkel, Kohane, and their colleagues know that they need to persuade very large numbers of patients to enroll in similar studies. Researchers around the globe will need to be able to share such data widely, comparing patients’ medical histories with their genetic profiles, while simultaneously recognizing Kohane’s concerns: that stockpiling vast quantities of intimate biological information—the key to this new kind of medicine—in turn raises new ethical dilemmas.

The Gene Partnership team foresees that when large numbers of people learn more about their risks of contracting specific diseases and their sensitivity to medications, they will likely engage in new kinds of conversations with their medical caregivers. One significant issue will be whether genetic-test results offer false alarms or hide real problems. This issue sharpened for Kunkel when a study of autism that he and Kohane were conducting seemed to show that two of the participating children had a mutation associated with leukemia. Kohane recalls losing “two nights of sleep” over whether or not to tell the parents. Although retesting the data revealed that the apparent leukemia link resulted from an experimental artifact, Kohane, Kunkel, and their co-workers began thinking about the practical details of a system that would convey risk information to patient-participants while maintaining privacy.

PATRICK LANDMANN/PHOTO RESEARCHERS, INC.



and treatment—even as the patient’s privacy is respected.

In a medical world with millions of patients participating in genetic research, prime goals of years and decades of cooperative study would be to tune treatments to people’s inherited characteristics, to keep track of whether people actually come down with a disease to which they are predisposed, and to unravel the true mixture of genetic and environmental influences on disease. In contrast to the usual practice in today’s genetics studies, participants would be able to retrieve their personal data if they want to, and receive genetic counseling. The aim is a new deal between volunteers and genetic researchers. Kohane and his colleagues feel that this new deal is both imperative and technically attainable—if researchers and physicians will acknowledge that participants are capable of processing complex medical information.

Kunkel, who serves as a principal adviser to the Muscular Dystrophy Association, a U.S. patient-advocacy group with a \$160-million annual budget, and also directs the genomics program at Children’s, has been a strong advocate for such a patient-

In the forelimb of an experimental mouse with DMD, orange and yellow areas indicate dead muscle fibers where dystrophin, indicated in green, has disappeared. After treatment with a type of gene therapy known as exon skipping, the mouse regained normal dystrophin levels and muscle function. The technique has not yet been tried in humans.

The group has raised these issues repeatedly in print. A 2007 article in *Science*, in particular, advocated “reestablishing the researcher-patient compact” by advocating what has become the Gene Partnership. They envisioned patients adding samples and information as they wished, or withdrawing from the cohort if they chose. The patients would receive their own medical records (as already happens at some healthcare facilities, including those operated by the Veterans Administration). Patient-participants would control when they were contacted by choosing when to “tune in” to alerts about discoveries and their potential clinical impact. These “broadcasts” aimed at the anonymous subjects of the Gene Partnership would incorporate carefully described characteristics

that recipients would recognize. The alerts might also include requests from the researchers for additional information or samples. To make the scheme work, Kohane and the others admit, would require tackling problems of low “health literacy,” lack of access to the Internet, and hammering out principles of what to tell participants and when.

In the last five years, through “genome-wide association studies,” researchers using DNA chips have uncovered several hundred genetic factors linked to common diseases—but most of these add only a small percentage to a person’s risk of a particular disease.

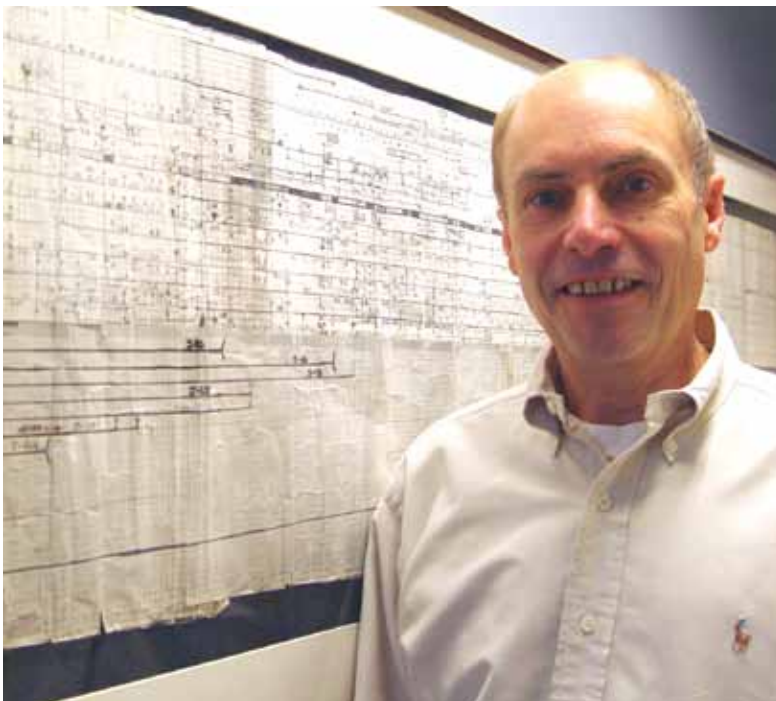
These plans are taking shape amid much skepticism about personalized medicine. Critics pointedly ask whether the readouts from people’s genomes (early commercial versions are now available) are truly medically useful. Common diseases are linked to several, even many, interacting genes, and to a complicated battery of controls that are just beginning to be studied. The functions that many genes specify are still not clear. In the last five years, through “genome-wide association studies,” researchers using DNA chips have uncovered several hundred genetic factors linked to common diseases—but most of these add only a small percentage to a person’s risk of a particular disease, and most are just neighbors of the real suspects. Attention is turning to the idea that the real villains are rare but “penetrant” mutations, still largely undetected, that require a more powerful “microscope” than the chips provide. Thus, complete sequencing of all or part of many individual patients’ genomes, at prices near that of a CAT scan, is looking more and more attractive, even though understanding the full impact of the genome on health lies in the future.

DESPITE the slow implementation of gene-based treatment in clinical settings, a firm belief in genomic medicine continues to drive both private and public sequencing research. The main unknown remains simply *when* large-scale changes will occur in medical care. Of course, this was also the case when the organism responsible for tuberculosis was discovered in 1882, 40 years before a countervailing vaccine, and 60 years before a countervailing drug, were developed.

The push toward medical applications looks difficult today not only because of the biological complexities of disease, but

Fixing Every Muscle

THE ARTICLE “Mother Courage: A family tragedy and a scientific crusade,” by John Colapinto, in the December 20, 2010, issue of the *New Yorker* (www.newyorker.com/reporting/2010/12/20/101220fa_fact_colapinto), describes Patricia Furlong’s efforts to raise public awareness of muscular dystrophy, and funding for research, and covers the trials of drugs designed to fight the disease. Both her sons were diagnosed with, and later died from, DMD. A cure is probably decades away. As Lee Sweeney, scientific advisor to Parent Project Muscular Dystrophy, the foundation Furlong created, told Colapinto, “It’s not just fixing one muscle. It’s fixing every muscle in the body. That’s the problem. Getting the cells to the right place and then getting them to do the right thing—it’s a daunting engineering problem as much as anything else.”



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also because of the structure and regulation of the pharmaceutical industry. The preferred pharmaceutical product is a “blockbuster” drug that can be used by millions—a market big enough to defray the vast investments of time and money needed to develop drugs and carry them through complex tests of safety and efficacy in animals and then humans. Rare diseases such as DMD are not profitable under this regime.

But this calculus may be overturned someday; research in genomics increasingly indicates that there is a genetic influence on the *effectiveness* of various drugs, from those used for cancer chemotherapy to blood thinners like Plavix. The trend toward what is called “genetic stratification,” a subject of increasing interest to the U.S. Food and Drug Administration, runs against blockbusters, pointing instead to a much more fragmented market in which drugs are tailored to the genetic characteristics of subgroups. In cancer treatment, for example, insurance companies may increasingly see the point of genetic testing if a \$50,000-a-year drug regimen works on only a subset of the patient population. Two major centers of cancer treatment, Massachusetts General Hospital and Sloan-Kettering in New York, have already begun implementing plans to extend genetic testing gradually to all their cancer patients, and partial or complete sequencing of a person’s genome may eventually become the gold standard for patient care.

The genetic basis of most diseases is still not fully known, but the finer sieve of whole-genome sequencing is already useful in diagnosing and addressing afflictions, like DMD, that involve rare but significant genetic changes. That is why the global genomic enterprise is exploding in numbers, dollars, research sites, and commercial commitment. Looking back at his career, Kunkel says simply, “The promise was there. I never said it would be easy.”

Victor K. McElheny ’57, NF ’63, is the author of the recently published *Drawing the Map of Life: Inside the Human Genome Project* (Basic Books).