5 When the Gene Is the Cure: Immunodeficiency and Gene Therapy

David Phillip Vetter could not live like this any longer. His doctors knew it; his parents knew it; he knew it. They all agreed he had to risk the bone-marrow transplant. Without it he would have to continue living in the bubble—his sterile isolation chamber—waiting for a cure to be developed for his affliction. Because David suffered from Severe Combined Immunodeficiency (SCID), he had no immune system to fight off even the most timid of invaders. He had already waited for twelve years, and still no cure for his condition was in sight. On October 21, 1983, he received some of his sister's bone marrow. It didn't take. Worse, it gave him cancer. He died February 22, 1984, 15 days after walking out of his bubble for the first time.

The first son of Carol Ann and David Vetter Jr. also began life with no immune system, and died of a massive infection six months after birth. His personal DNA code included an X chromosome, inherited from his mother, that carried a defective copy of the gene called *IL2RG*, which provides the instructions to make a protein required for the immune system to develop properly. Because there was a mutation—a change in the DNA sequence—in the *IL2RG* gene David Joseph inherited from his mother, the gene directed the production of a nonfunctional protein. Without the IL2RG protein, David Joseph's thymus, a small organ near the lungs where immature white blood cells from the bone marrow bivouac before going into battle, could not send off white cells to fight infections.

After their experience with their first son, Carol Ann and David Jr. understood that if their next child were a son, he would also have a 50 percent chance of being born with no immune system. A son has only the single X chromosome he inherits from his mother, his other sex chromosome being the Y chromosome he inherits from his father. So if one of the genes on the X chromosome were defective, he would suffer the consequences

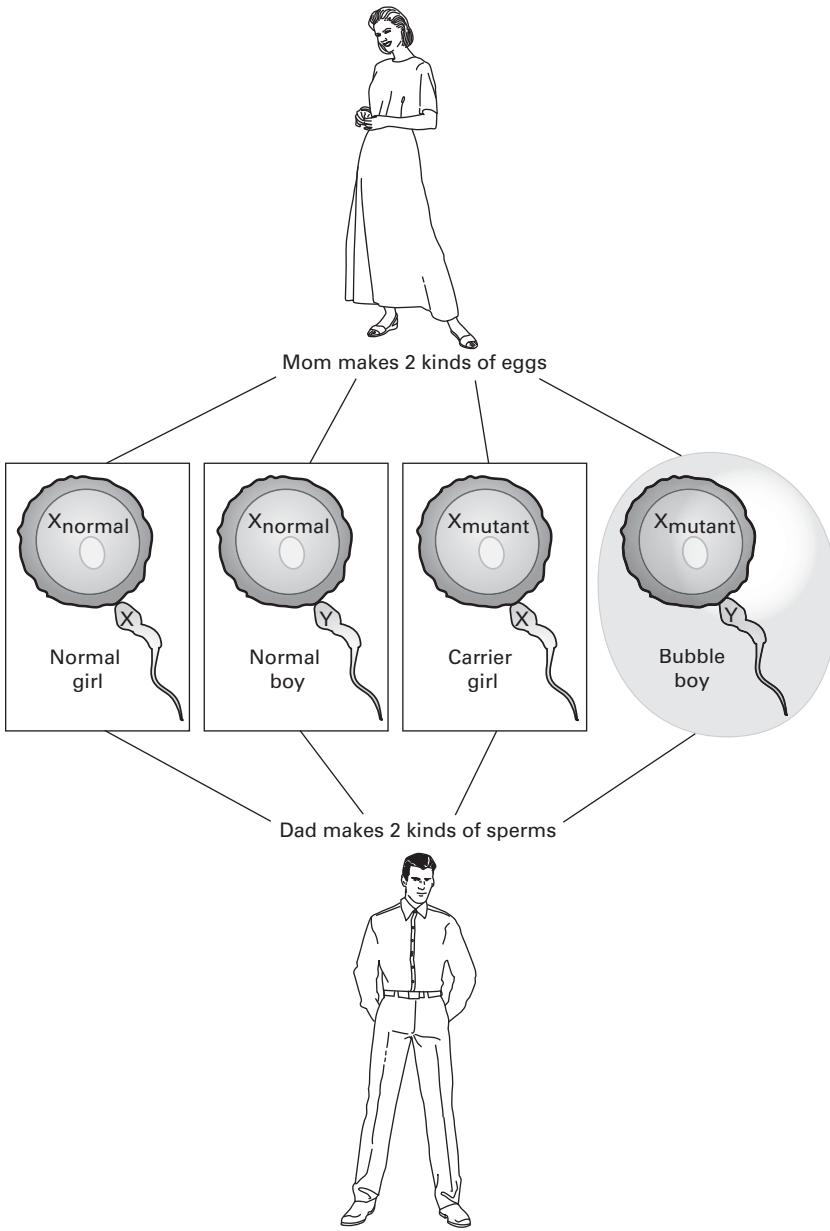
(see figure). A daughter would be safe, because even if Carol Ann gave her the X chromosome with the defective gene on it, her father, David Jr., would provide another X chromosome carrying a good version of the gene. (In chapter 7 we discuss in more detail why one good gene may be all you need.)

Diseases like SCID that are due to a defective gene on the X chromosome are passed to boys only from their carrier mothers, who have a good version of the gene on their other X chromosome. Males with the mutant gene on the X die of the immune disease before they are old enough to reproduce and pass the flawed X chromosome on to their daughters.

But the Vetters were told that even if their next son were unlucky and drew the defective chromosome, he would not necessarily be doomed: The doctors thought they could cure his disease, either with a bone-marrow transplant from his sister or with a cure they thought was just around the corner. They had on their team Dr. Raphael Wilson, an expert in germ-free environments, who would build and maintain the sterile isolation chamber—the "bubble"—that would protect their infant son from the germs that had killed his brother.

A few years before, Wilson had reported stunning success in Germany with a sterile isolator he built for twins with immunodeficiency: after a short time in the bubble their immune systems suddenly, and inexplicably, came to life, and the twins were taken out of the isolator. So the Vetters' doctors were optimistic. Wilson "just swept us along with his enthusiasm. He had the confidence to say, 'We can do this. We can do this,'" said Dr. Mary Ann South, one of the members of the medical team, in a documentary film about the child who became known as the Bubble Boy.

Carol Ann and David Jr. were eager for another child. Although they had a healthy girl, they wanted a boy to carry on the Vetter family name. "Children were very essential to our hope and to our dream of the future. We wanted to have children right away; we wanted to have as many as God would send us," Carol Ann explained in the TV documentary. So they had another child. Happily, one of their dreams came true: it was a boy. Sadly, their other dream did not: the boy did not inherit his mother's functional *IL2RG* gene. Instead, like his older brother, he inherited her X chromosome that carried the defective gene. He was whisked into the isolator within seconds of his birth, and that's where he stayed for twelve years, until it became obvious that no cure was imminent.



David Phillip Vetter, the Bubble Boy, lived a celebrated life that stimulated a hit song by Paul Simon, feature films starring John Travolta and Jake Gyllenhaal, and an episode on *Seinfeld*. Celebrated, but tragic. The journalist Steve McVicker described in a 1997 article in the *Houston Post* how David responded when his friend the psychologist Mary Murphy asked him why he was so angry: "Why am I so angry all the time? Whatever I do depends on what somebody else decides I do. Why school? Why did you make me learn to read? What good will it do? I won't ever be able to do anything anyway. So why? You tell me why!" Murphy had no answer for David Phillip.

Had David Phillip Vetter been born twenty years later he might have chosen to wait a little longer, because in the year 2000 a cure for SCID finally became available. Not a perfect cure, but there can be little doubt David would have jumped at the chance to try it. The cure comes in the form of the good *IL2RG* gene—the gene whose lone copy on David's lone X chromosome didn't work.

If a functional copy of the gene can be delivered to the bone-marrow cells of a SCID patient, those cells begin to make white blood cells competent to fight infections, giving the patient something he wasn't born with: a functional immune system. Treating disease with genes—gene therapy—is the brass ring that David and his parents and his doctors were waiting for. It cured the disease for thirteen boys in France and England. But gene therapy came too late for David.

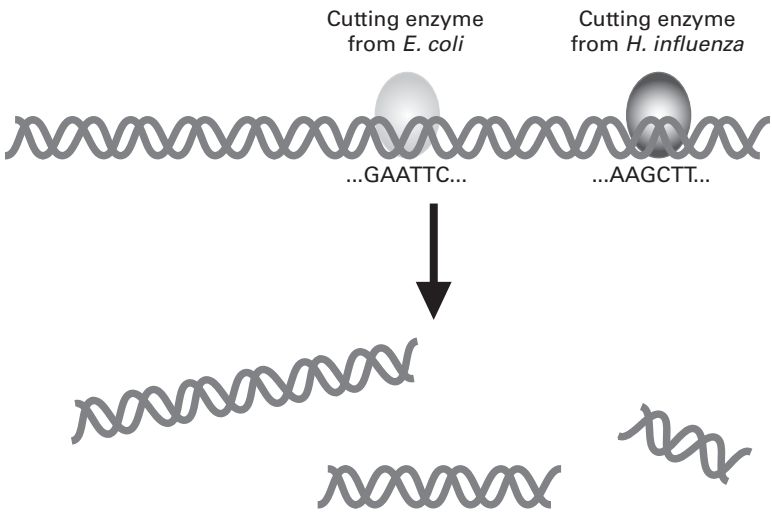
What occurred after David's death in 1984 that made gene therapy a viable treatment for his disease by 2002? Lots. The human genome was mapped, making it possible by 1987 to identify the region of the X chromosome that carries the *IL2RG* gene. These maps, as we'll discuss in chapter 12, show the positions of genes along a chromosome, just as roadmaps show the positions of cities along a highway. By 1993 scientists had isolated the *IL2RG* gene, using methods for isolating genes developed in the 1970s. In the 1990s scientists devised methods to deliver genes to human cells, so by 1999 they could deliver the *IL2RG* gene to the bone-marrow cells of five children with SCID. By 2002 it was clear that most of these children were cured: four have a nearly normal immune system and are enjoying what David longed for: a life outside the bubble.

How, exactly, was all of this done? Once a gene is located on a chromosome, how is it purified and isolated in the test tube? The principle

is simple: the chromosomes are fragmented into small pieces of DNA, and the piece containing a particular gene is fished out of the mixture and copied millions of times, in a process called cloning. It's like making copies of an animal, as was done to clone the sheep Dolly, but in this case multiple identical copies (clones) of the gene are made from a pure template. Each copy is a clone of the original gene that provided the template.

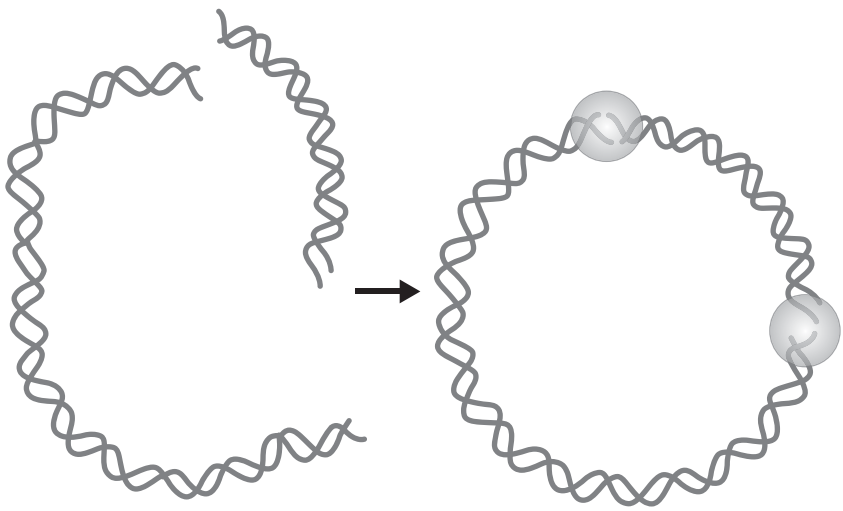
Gene cloning is not much different from what you do when you include a passage from Shakespeare in your wedding announcement. You open your massive compendium of the bard's plays and search through it, page by page, until you find the specific sequence of letters you desire: "Doubt that the stars are fire; Doubt that the sun doth move; Doubt truth to be a liar; But never doubt I love." You extract that passage, insert it into your announcement card, and make many copies of the card to send to friends and family. You have cloned a passage from *Hamlet*, act II, scene ii.

Because of remarkable technical advances of the 1970s, it's now almost as easy to find and copy genes as it is to find and copy passages from a book. The first step is to chop all the chromosomes into small pieces, which can be accomplished by adding to the chromosomes enzymes that cut DNA. Those enzymes don't cut the DNA just anywhere. They recognize specific short sequences of bases in DNA and cut wherever those sequences occur, producing a discrete set of fragments (see figure).



These enzymes are obtained from bacteria, where they provide defense against invaders such as viruses that infect bacteria by cutting up the viral DNA to prevent the viruses from commandeering the bacterial cell. Each species of bacteria has a unique set of these enzymes, and each enzyme cuts DNA at a different sequence of DNA base-pairs. For example, an enzyme in the common gut bacterium *Escherichia coli* cuts DNA wherever it finds the base sequence GAATTC on one of the strands; an enzyme from the bacterium *Hemophilus influenzae*, which causes pneumonia, cuts DNA wherever it finds the base sequence AAGCTT. Over three thousand of these enzymes have been characterized, and most are readily available, allowing gene hunters to divvy up the chromosomes into bite-sized pieces—basically dividing the book that is our genome into paragraphs.

Having cut up the genome, we need to separate all the fragments—there are millions of them—much as we separate the pieces of a jigsaw puzzle before starting to piece them back together. The fragments are first inserted, one by one, into a minichromosome, a small circular piece of DNA derived from a bacterial chromosome, using another kind of enzyme that joins two pieces of DNA, as an ironworker constructing a skyscraper joins two steel beams (see figure). All cells have such a DNA-joining enzyme because they constantly need to unite pieces of DNA to repair the damage that DNA continually incurs.



The minichromosomes are slipped back into bacteria, which act like little copying machines to make copies of the minichromosomes that carry a piece of a human chromosome. Each bacterial cell divides to produce a colony of billions of cells, each carrying a minichromosome containing a particular piece of the human genome. Among those millions of identical-looking colonies of bacteria, we need to identify the one that carries the minichromosome with the fragment that includes the gene we want. How can we do that?

We know the DNA sequence of the *IL2RG* gene because the base sequence—the precise order of A's, C's, G's, and T's—of the human genome has been determined, so the process now is simple: the base-pairing principle worked out by Watson and Crick means that any DNA strand will match up to its complementary partner strand through the specific plugs and sockets on each base. For instance, the sequence AGGCTAAC will match up to TCCGATTG because A pairs with T, and C pairs with G. So we can design and have synthesized a piece of DNA with a sequence of bases that matches up to some sequence in the *IL2RG* gene. Such synthetic DNA molecules can be ordered from providers of scientific supplies for not much more than you'd pay for a T-shirt. Because that piece of DNA will stick to the *IL2RG* gene, it can be used as a "probe" for the gene. We will attach to that probe a molecule that can be easily detected, like a compound that is fluorescent. When we spread some of this fluorescent DNA on top of the millions of bacterial colonies, it will find its mate only in the rare colony of bacterial cells that carries the *IL2RG* gene on its minichromosome, and the probe will literally "light up" that cell. We can then recover the cells of that glowing bacterial colony and retrieve the minichromosome from them, with not much more difficulty than we retrieve copies from the output tray of a Xerox machine.

The same enzymes that enable gene isolation spawned the biotechnology industry, today a worldwide enterprise that generates over $50 billion in yearly revenue. This new industry has provided such drugs as erythropoeitin (Epo) and granulocyte colony stimulating factor (G-CSF) for stimulating the growth of blood cells in patients who have undergone chemotherapy for cancer, and antibodies such as the one that fights breast cancer by inhibiting the Her2 protein, and insulin for diabetics, and Etanercept for treating disorders of the immune system such as arthritis

and psoriasis, and the blood-clotting Factor VIII for hemophiliacs, and many more.

In addition to enzymes that split apart and splice together DNA molecules, there are enzymes that can duplicate DNA sequences to make more copies of them, enzymes that can change one sequence to another, and enzymes that carry out some of the many steps required to manufacture pharmaceuticals. None of these enzymes was sought by biologists to create an industry. Rather, they were discovered—quite fortuitously—in scientists' quest to understand how bacteria fight infection, or how they replicate their DNA, or how they synthesize their proteins, and many other seemingly esoteric questions. Clearly, basic research is a good value.

Soon after he was born in Buckinghamshire, England in August 2003, Alexander Locke was diagnosed with the same disease that killed David Joseph and David Phillip Vetter. Alexander's parents, Carol and Colin Locke, like the Vetters, had no idea their firstborn son was at risk of having SCID. "We realised Alexander had a problem when his tummy button inexplicably failed to heal after birth, despite repeated courses of antibiotics. At four months, he developed a severe viral respiratory infection. He spent his first Christmas in hospital, attached to oxygen lines and antibiotic drips," Colin told Andrea Kon, a reporter for England's *Daily Telegraph*. "He had inherited his defective X-gene from me," said his mother, "and it was hard to accept that it was my 'fault.' I had no idea I carried a 'bad' X-gene."

Alexander was put in an isolator in London's Great Ormond Street Hospital for Children. It was more comfortable than David Phillip Vetter's bubble because the technology had improved in the intervening twenty years: Alexander had an entire room to romp around in. Alexander was protected by an airlock through which all his visitors had to pass in order to have the air around them cleaned and filtered. He was to live in the isolator while he waited for his doctors to identify a perfectly matched bone-marrow donor, something for which David Phillip Vetter waited for in vain for twelve years.

But Alexander spent only eight months in the isolator. Drs. Adrian Thrasher and Bobby Gaspar at Great Ormond Street Hospital were getting ready to test an experimental gene therapy for treatment of SCID, and when Alexander's bone-marrow transplant fell through (because the nearly

perfectly matched donor carried a virus that would almost certainly have killed him), Alexander entered the gene therapy trial, along with four other boys with SCID.

Drs. Thrasher and Gaspar had developed a vehicle to deliver to Alexander's bone-marrow cells a good version of his defective gene. The vehicle was a virus that infects human cells. Viruses are ideal for this job because they are basically tiny Trojan horses that carry DNA within their protein coat. The viral DNA contains genes, just as our DNA does, and these genes code for the viral proteins that make up the protective coat and that make copies of the viral DNA. Although the viral DNA is minuscule compared to ours—most viruses have just a handful of genes and often only a few thousand DNA base-pairs—scientists have found places in this DNA where other genes, human genes, can be inserted.

The virus enters a cell and removes its coat, thereby delivering its DNA inside the cell. If it's a normal virus, the cargo is the viral chromosome with its genes that encode proteins to commandeer the cell's machinery to make more virus. Some viruses are aggressive, making many copies of themselves and killing their host cells in the process, releasing more viruses that go off to infect and kill other cells. Other viruses are relatively benign, incorporating their own DNA into a human chromosome while allowing the cell to live, lying in wait to check out the situation before deciding to make more virus. But if some of the viral genes are removed from its chromosome, the virus is disabled: it can deliver its DNA into cells, but that incomplete viral chromosome cannot take over the cell or produce more virus.

Drs. Thrasher and Gaspar spliced the *IL2RG* gene into the chromosome of a disabled virus that infects human cells. They made many copies of the engineered viral chromosome, packaged them into viral coats, and added the viruses to a test tube containing Alexander's bone-marrow cells. The viruses latched on to the marrow cells and quietly slipped into them, taking off their viral coats as they went in.

The viral DNA made its way to the cell's nucleus where it pasted itself along with the *IL2RG* gene into one of the human chromosomes. As the cells grew and divided they passed the viral DNA along with the good *IL2RG* gene on to other bone-marrow cells. The engineered cells were returned to Alexander's bloodstream, where they found their way back to his bone marrow.

Many processes have to go right for gene therapy to work, and scientists are still a long way from having a failure-proof procedure. For gene therapy to work, biologists need to deliver the viruses carrying the therapeutic genes to the appropriate cells, where the good gene can do its job. Discoveries of how cells specialize in certain tasks have led to improvements in this cell targeting. Even if the good gene gets to the right cells, it must get turned on at the right time and at the right level to provide cells with the right amount of the protein they are missing, when it is needed. Furthermore, expression of that gene must persist for long periods of time, so understanding how transcription factors and other proteins determine whether a gene is on or off is invaluable.

Drs. Thrasher and Gaspar waited to see if the engineered bone-marrow cells would give rise to the white blood cells Alexander needed to fight infections. "Alexander was allowed home for the first time in May, aged eight months. It was more complicated than having a newborn," Carol told Andrea Kon. "Every tube and piece of equipment needed sterilising. We had to use a spreadsheet to keep track of his medical regime. He had lost the ability to suck during the first days in intensive care and was being fed through a gastronasal tube. We were administering four drugs four times a day through the tube."

Despite their best efforts, Alexander contracted infections and had to be rushed back to Great Ormond Street Hospital. "The second time doctors fought a nine-hour battle for his life; we were so lucky. It's astounding he didn't suffer any long-term brain damage," Colin exclaimed.

Alexander's new bone-marrow cells grew and began to spawn competent white blood cells. He was allowed to venture out of his airlocked room for longer and longer periods of time. "He loved mixing with other children, although he had never played with a child until six months ago," his mother said. "We feared that his life as a 'bubble baby' might have left developmental or physical scars, but he's caught up in every way. The only treatment he needs now is a prophylactic course of antibiotics once a fortnight. Soon he'll go to primary school. That would have been unthinkable two years ago." The cure that David Phillip Vetter was waiting for finally arrived. It came in the form of a gene.

But good as it seems, the cure is far from perfect. The virus that delivers the *IL2RG* gene to bone-marrow cells of SCID sufferers can also deliver

something deadly: cancer. Four of eight boys in France who were cured of SCID by gene therapy traded it for another disease: leukemia. One has since died from it. The cancer occurred because the piece of viral DNA that carries the *IL2RG* gene, which usually inserts itself benignly into apparently nonfunctional regions of the genome far from any critical genes, landed in these boys' genomes near a gene encoding a protein that accelerates cell growth. The viral DNA caused this gene to be turned on, and the protein it made set those cells on the path to cancer.

The doctors had no way of knowing in advance that the virus would land near this gene, but as soon as they learned that it had, they stopped doing gene therapy with the virus while they searched for a way to prevent it from happening again. Is the prospect of a cure for their disease worth the risk of leukemia for these boys? We suspect that David Phillip Vetter would have said it is.

It is not surprising that the only real successes of gene therapy since David Phillip Vetter's death in 1984 have been with disorders, like SCID, that affect cells that doctors can easily get their hands on. Surgeons are good at harvesting bone-marrow cells from patients, and scientists are adept at growing them and modifying them in the laboratory. And it is easy to get the engineered cells to the place they are needed, because when they are reintroduced into the bloodstream they find their way back to the bone marrow, like salmon returning to their home river to spawn.

Other attempts at gene therapy have not been so successful. Dr. Ronald G. Crystal at the U.S. National Institutes of Health had a great idea for delivering to patients a good copy of the gene that is defective in people with cystic fibrosis. This gene encodes a protein that sits in the membrane of lung cells and allows salt to pass in and out. If the protein is defective and the salt balance is upset, then the layer of mucus that keeps germs out of the lungs becomes thick, providing an attractive breeding ground for infectious bacteria. The inflammation of the airway that results makes breathing difficult. Cystic fibrosis is a disease that usually leads to an early death.

Crystal reasoned that he could deliver the gene to patients' lung cells simply by having them inhale disabled viruses that carry the cystic fibrosis gene. The viruses would be sucked into the lungs, where they would attach

to cells and inject the functional gene. He used a relatively harmless virus that naturally infects the lungs and gives people mild cold symptoms. While the idea seems terrific, it didn't work because the lung cells are protected by an armor of mucus and cilia, little hairs that sweep away foreign particles that enter the lungs, which ended up blocking access of the viruses to the cells.

One gene therapy failure in 1999 was especially tragic. Jesse Gelsinger, at eighteen, suffered from a metabolic disorder caused by the lack of the ornithine transcarbamylase (OTC) enzyme, which is needed to prevent a toxic product of metabolism, ammonia, from accumulating in the blood. The accumulation can lead to brain damage, coma, even death. Jesse had a mild form of the disease, which he was able to keep under control with medication (he took thirty-two pills a day) and a low-protein diet. He knew gene therapy was unlikely to help him, but he was eager to try it because it promised to help those with more severe forms of the disease. Jesse told Sheryl Gay Stolberg, a reporter for the *New York Times*, "What's the worst that can happen to me? I die, and it's for the babies."

At 10:30 a.m. on Monday, September 13, 1999, a large dose of a virus carrying the *OTC* gene was injected into a vein that emptied into Jesse's liver. The plan was that it would deliver the gene to his liver cells, which would then make the enzyme he needed. We'll never know if that happened, because Jesse died four days later, the victim of a massive reaction of his immune system to the virus. His death cast a pall over gene therapy for several years.

Given its few successes and its several failures and tragedies, gene therapy has yet to live up to its much-ballyhooed potential. There are still enormous challenges in getting functional versions of genes into the right cells and, once there, getting them to produce an appropriate amount of the needed proteins for long periods of time.

And gene therapy brings enormous ethical questions. If we can change someone's personal DNA code by replacing a defective *IL2RG* or *OTC* gene in order to cure a disease, we could probably change a gene to make a child taller, or stronger, or have a lower level of cholesterol, or concentrate for longer periods of time. What are the limits on human characteristics that are permissible to alter? And what about making changes to the DNA code that would be passed down to future generations? Although there is general agreement that this type of gene therapy should never be attempted,

human history suggests we must remain vigilant. Scientists are nothing if not persistent and ingenious, and they have no lack of alternative strategies to someday bring gene therapy into standard practice. But the expectant public that has learned of the potential of gene therapy to relieve suffering from diseases, as well as scientists themselves, must be patient. For David Phillip Vetter the wait would have been twenty years for a possible cure; for other patients with other diseases, the wait will be longer. But the cures *will* come. Of that we are confident.

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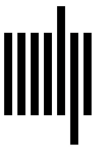
By: Stanley Fields, Mark Johnston

Citation:

Genetic Twists of Fate
By: Stanley Fields, Mark Johnston
DOI: 10.7551/mitpress/8709.001.0001
ISBN (electronic): 9780262289382
Publisher: The MIT Press
Published: 2013



The MIT Press

This book was set in Stone Sans and Stone Serif by Toppan Best-set Premedia Limited.
Printed and bound in the United States of America.

Library of Congress Cataloging-in-Publication Data
Fields, Stanley.
Genetic twists of fate / Stanley Fields and Mark Johnston.
p. cm.
Includes bibliographical references and index.
ISBN 978-0-262-01470-0 (hardcover : alk. paper) 1. Medical genetics—Popular works. 2. Human genetics—Popular works. I. Johnston, Mark, 1951– II. Title.
RB155.F54 2010
616′.042—dc22

2010006926

10 9 8 7 6 5 4 3 2 1