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by Jean S. Helgeson

Stormie Jones was a thirteen-year-old girl from White Settlement, Texas, who just wanted to grow up and be normal. She did not get to do either. On November 11, 1990, she died when her body rejected the transplanted heart that had kept her alive for over six-and-a-half years. North Texas and the world mourned her death, for she was a fighter and a pioneer, but she simply could not overcome her main obstacle. She was a homozygote for familial hypercholesterolemia, with two copies of a single gene that gave her extremely high blood cholesterol.

In 1984 a medical first occurred that was related to the work done over the previous twelve years in a laboratory at Southwestern Medical School in Dallas. Stormie, then a six-year-old child who had already had two heart attacks caused by her severely elevated blood cholesterol levels, was the first recipient of a combined heart and liver transplant. Because of work done in Dallas, it had been known that a liver transplant should accompany the replacement of her damaged heart. The liver from a normal donor had receptors for low density lipoprotein (LDL) on its cell surfaces that could clear out much of the cholesterol from her blood, something that could not be done by her own liver. The combined replacement of both heart and liver allowed Stormie to live until age thirteen, well past the time when she would have died without the transplants.

Doctors Michael Brown and Joseph Goldstein, of the University of Texas Southwestern Medical School in Dallas, jointly received the Nobel Prize in Physiology and Medicine in Stockholm, Sweden, on December 10, 1985, twenty-two months after Stormie's pioneering surgery. They were quite young for this recognition, only 44 and 45 years old, respectively. This most prestigious international award in science was given to them for their innovative collaborative work since 1972 in the study of cholesterol metabolism. Because of that work, these physicians had known that a liver transplant was a necessary accompaniment to the required heart transplant as the best chance for giving Stormie a relatively normal liver.

Cholesterol has been important in medical and scientific studies since its discovery in gallstones over two-hundred years ago. Besides forming gallstones, this lipid is involved in the production of atherosclerosis, a form of hardening of the arteries, through its deposition in the walls of damaged blood vessels. Cholesterol is not only involved in causing harm to the body, however. It is required in the cell membranes of all animal cells, and is the main component of steroid hormones such as estrogen, testosterone, and cortisone. An inherited form of high blood cholesterol was discovered in 1938 and named familial hypercholesterolemia, abbreviated FH. The study of the inherited aspects of cholesterol metabolism by Brown and Goldstein has led to greatly increased understanding of the body's regulation of the amount of cholesterol it produces, as well as of the basic biological processes of cellular function and genetic control.

While mammalian cells are capable of making their own cholesterol, they also obtain it from the diet through delivery of low density lipoprotein, or LDL, by the blood from the digestive system to the tissues. The cholesterol molecule is relatively insoluble in water and must be moved through the bloodstream in a coating of the more soluble lipoprotein. An equilibrium between cholesterol made by the cell and that obtained from the diet was recognized in the 1930s, but its mechanism was not understood until it was explained by Goldstein and Brown. They discovered that LDL delivers cholesterol to the body cells by binding to a protein receptor on the cell surface, the LDL receptor, which then is carried into the cell. The genes and proteins involved in the regulation of the pathway of LDL and cholesterol metabolism have been extensively explored by these two scientists and their associates.

Brown and Goldstein had made their first and probably most major discovery in 1973. They found that a key enzyme in cholesterol production, HMG CoA reductase, could be regulated in culture in normal cells by the amount of low density lipoprotein in the culture medium, but regulation was absent in cells of homozygous FH patients. In normal cells, the addition of LDL caused the enzyme to be turned off, and the cells would stop making cholesterol. In homozygous FH cells, even high levels of LDL could not turn off the enzyme, so cells continued to make cholesterol even when extracellular levels were very high. The level of regulation in heterozygotes, the parents of the homozygotes, was intermediate between that of the normal cells and the homozygotes' cells. From this it appeared obvious that the defective gene must deal with the uptake of LDL and its entry into the cell, where it would be able to regulate the HMG CoA reductase. The defective gene was believed to produce a receptor protein that would bind LDL and allow it to enter the cell. This belief was further strengthened by the finding that cholesterol added to the cultures in alcohol, rather than in LDL, could enter the cells of both normal and homozygous cells and cause the HMG CoA reductase to be turned off. The defect was not in the enzyme, but in the delivery of cholesterol to the inside of the cell. The experiments done to test this system were well-designed and well-executed, giving clear-cut results. The level of excitement in the laboratory and among the faculty of the medical school was high. It was obvious even in 1973 that this discovery was of major proportions, the first proof of a mechanism for a genetic disease that involved a faulty cell-surface receptor.

Their discovery was recognized immediately for its quality and importance, leading to national prominance as early as 1974, when the so-called "gold dust twins" won the first of many awards in recognition of their lipid research. Four national and international awards in the 1970s were followed by twelve others in the 1980s, besides the Nobel Prize. President Ronald Reagan in 1988 awarded Brown and Goldstein the National Medal of Science, the nation's highest award for scientific achievement. Goldstein was selected as a member of the Program Advisory Committee on the Human Genome for the National Institutes of Health, overseeing one of the major international scientific efforts of the 1990s, the determination of the genetic make-up of the human.

The two young men had met during their internship in internal medicine at the Massachusetts General Hospital in Boston in 1966, after Goldstein had graduated from Southwestern Medical School in Dallas and Brown from the University of Pennsylvania Medical School. Their friendship continued when both went on to fellowships at the National Institutes of Health in Bethesda, Maryland, in 1968. Goldstein convinced Brown to come next to Southwestern Medical School, where Goldstein had been offered the future position of head of medical genetics while he was still a student. Brown began working in gastroenterology at Southwestern in 1971, and Goldstein arrived in 1972 after spending two years studying medical genetics in Seattle, Washington. They began an immediate collaboration that continues into the 1990s, dealing with patients with genetic lipid disorders and the biochemistry of those disorders.

Goldstein had first seen patients with familial hypercholesterolemia at Bethesda. This disease usually causes the presence of very high levels of cholesterol in the blood, leading to atherosclerosis, skin deposits of

cholesterol, and heart attacks. In Dallas, Goldstein and Brown set up a tissue culture laboratory and grew cells taken from the skin of normal persons and the skin of patients with FH. The cells were analyzed biochemically after being treated with different protocols, and metabolic activities in the isolated cells were recognized to be the same as those in cells in a patient's body.

An important aspect of this research was that Goldstein and Brown had access to several patients with extremely high cholesterol levels. These were the young children of parents who each had one mutant gene for high cholesterol, and the children had received a defective gene from each parent. This condition happens once in a million people, producing FH homozygotes like Stormie. Cholesterol levels in homozygotes often surpass 1000, producing heart attacks by the age of four or five, and usually death before age fifteen from heart damage. The single defective gene occurs about once in 500 people, producing FH heterozygotes who have high cholesterol levels around 250-600, and tend to have heart attacks around age forty. A "normal" cholesterol level is around 120-220, although even persons without the FH gene can have higher levels that lead to heart disease, and most heart disease does not involve familial hypercholesterolemia.

In the laboratory, Brown and Goldstein found that receptors for LDL localized to particular spots called coated pits on the cell surfaces of normal skin cells, while no LDL receptors occurred in coated pits on cells of homozygotes for FH. Coated endocytic vesicles, small sac-like structures that pinch off from coated pits and move into the cell's cytoplasm, were found to carry LDL into the cytoplasm in normal cells, but again to contain no LDL in FH homozygote cells. The coated vesicles bind to lysosomes, sac-like structures that contain digestive enzymes within the cytoplasm, and the lysomal enzymes digest the LDL. This makes cholesterol available to the cell and causes the enzyme HMG CoA reductase to be turned off, so no more cholesterol is produced by the cell. The LDL receptors were shown to be recycled to the cell surface every ten minutes for reuse in normal cells.

A new kind of defect was found in the LDL receptor binding and internalization pathway. An FH homozygote known as J. D. was discovered to be able to bind some LDL to his cell surfaces, but the LDL could not localize to the coated pits to be taken into the cell, as was seen in normal cells. One of his parents had the standard mutation for FH, in which no receptors were made, while the other parent had the new mutation, which produced receptors that could bind LDL but could not localize to a coated pit on the cell surface and go into the cell. With both mutations, J. D. thus had no LDL entering his cells, and so produced a high level of cholesterol. The new defect was in the internalization of the LDL receptors.

The multiple mutations in the LDL system were found to cause different disruptions of the pathway of transport and processing of the LDL receptor within the cell, as first seen with J. D.'s cells and later extended to other areas of the pathway when new mutations were discovered. The genes for these forms of mutant receptor proteins were sequenced and described. Each different mutation, as shown in various family groupings, contained individual changes in the sequence of the DNA nucleotide subunits in the gene, or perhaps a loss of large portions of the gene itself. Cells from over 110 homozygous FH patients have been studied and are still being sequenced, showing different kinds of mutations in different gene regions. In one patient, the LDL receptor was found to be secreted from the cell rather than bound to the cell membrane. In another family, a new dominant gene was found which suppresses the expression of hypercholesterolemia although there is also a gene for defective LDL receptors. This could be an important discovery for FH patients, since the understanding of how this new gene works could lead to new methods of treatment for FH and for non-inherited high cholesterol levels.

The human LDL receptor system was not the only one being studied by Brown and Goldstein. Metabolism of the pathway was also explored in the liver and adrenal gland of the rat. The liver is where cholesterol is made into bile for lipid digestion, and the adrenal glands are a site of steroid hormone production, so both have high levels of LDL receptors. Fresh adrenal glands from cattle were used to provide large amounts of tissue for LDL receptor purification studies,

which eventually resulted in the purification and identification of the bovine (cattle) LDL receptor protein. A modified copy of the gene itself was produced in the bovine system by using a bacterial enzyme, reverse transcriptase, allowing production of the receptor protein in large quantities for study in the laboratory. Earlier rabbit studies were broadened when a mutant strain, called WHHL rabbits, was developed in Japan from a mutant that showed LDL receptor deficiency. This provided an animal model of familial hypercholesterolemia. In November, 1990, Goldstein and Brown reported that genetic engineering had enabled them to replace the mutant FH gene with a normal gene in living homozygous rabbits. These animal studies obviously open up a range of opportunities for future human treatments for this and other genetic diseases.

Basic research often leads to medical applications, as it did in this laboratory. A chemical called compactin, derived from a fungus, was used in studies on the LDL pathway. Compactin acts as an inhibitor of the enzyme HMG CoA reductase, and turns off the internal synthesis of cholesterol in cells exposed to it. Cells could be made dependent on cholesterol delivered by the LDL receptors, and it was hoped that a drug might be derived from compactin that could be used in heterozygotes and others to lower their dangerously high blood cholesterol levels. Later studies showed that another fungus-derived chemical, mevinolin, could do the same thing better in living organisms. Mevinolin was marketed in 1987 as lovastatin, now a major treatment for controlling blood cholesterol levels in hundreds of thousands of patients worldwide. The life-saving drug is the direct result of work done in Goldstein and Brown's laboratory.

Emphasis in the laboratory has recently centered on the molecular level of genes, proteins, and membranes. As the human LDL receptor gene was sequenced, its various regions, or domains, were examined. One domain was shown to be essentially the same as the gene sequence for epidermal growth factor, which causes the growth of cells lining the inside and outside of the body. This growth factor is taken into cells through a pathway similar to the internalization of LDL receptors. The gene for the LDL receptor was shown to be a mosaic of domains that are shared with other genes and expressed by them in making proteins. The finding of these shared domains has led to greater understanding of the molecular level of evolutionary processes that allow new proteins to develop. The overall understanding of how the normal cell function has also been dramatically enlarged by this effort. The observation of biochemical changes in the proteins and the clinical expression of the disease in patients with various mutant genes has been extremely important in the development of understanding of how membrane proteins work and how the receptor recycling of membranes occurs.

Additionally, new insights have been gained in the treatment of one of the most common and deadly diseases of our time, atherosclerosis. Treatment has changed as a result, not only in cases of FH, but also in people who have "normal" LDL receptors but high levels of blood cholesterol. Brown and Goldstein have theorized that the elevated LDL levels in "normal" people are due to acquired deficiencies in receptor production and activity, induced mainly by a high-fat diet rather than by inherited defects. Since it is thought that as many as half of all Americans have cholesterol levels high enough to cause atherosclerosis and heart disease, an understanding of the mechanisms of entry of cholesterol into cells and of how to regulate them is of vast importance to the majority of Americans and others in industrialized countries where cholesterol levels are high. Perhaps it will also be possible eventually, through these studies, to spare other FH homozygous children the problems Stormie Jones had to face with her transplanted heart and liver, and her early death.

The author worked in Brown and Goldstein's laboratory between 1973 and 1980, and has several publications with them on cholesterol metabolism.