Winter 1984

Cholesterol-Watching II: Genes: [Dr. Jan L. Breslow]

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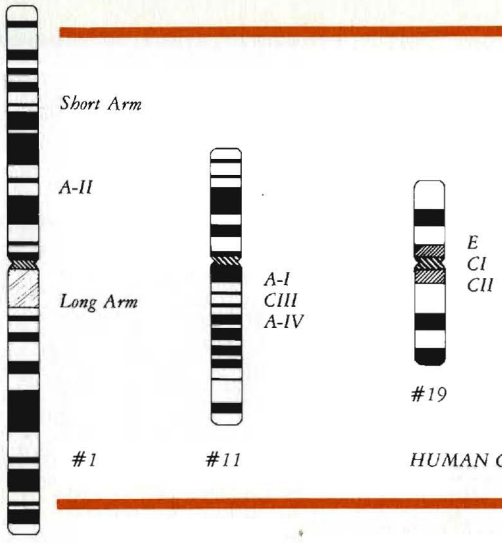


THE ROCKEFELLER UNIVERSITY
RESEARCH PROFILES

HUMAN CHROMOSOMES CONTAINING APOLIPOPROTEIN GENES

WINTER 1984/85

Cholesterol-Watching II: Genes

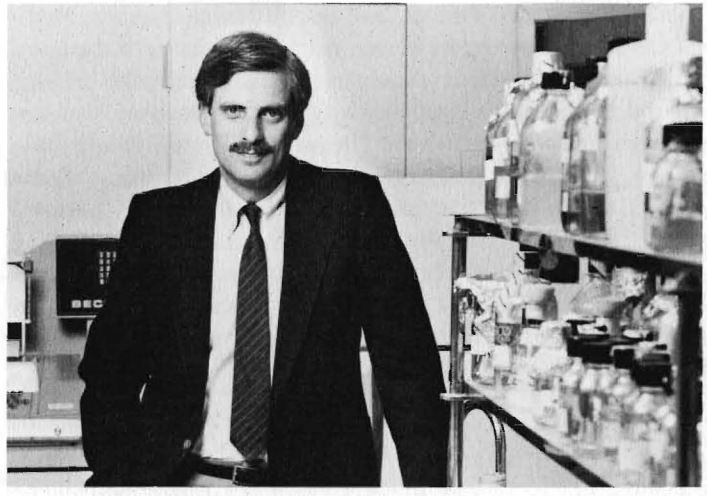
In a reception room at The Rockefeller University Hospital, a patient watches a videotape. On it a doctor is speaking.

"You are now in the outpatient clinic of the laboratory of biochemical genetics and metabolism. You are here because you or someone in your family has a problem with elevated cholesterol or cholesterol imbalance. Your doctor may have described your condition as hyperlipidemia, which means high levels of lipid, or fat, in the blood."

The tape, written and narrated by Margo Denke, a member of the laboratory's clinical staff, is a brief introduction to the role of cholesterol in the development of atherosclerosis, one of the thirty or so conditions under study in the hospital, which is Rockefeller University's clinical research center.

A drawing appears on the screen. Over the image, Dr. Denke's voice continues: "Here is a picture of a normal artery. Notice that the walls are thin and the opening is large. (The picture begins to change shape.) In the process of atherosclerosis, a yellow, fatty deposit, primarily cholesterol, accumulates on the inside of the artery making the walls thicker and the opening smaller. Eventually this buildup may completely block the opening and cut off the flow of blood. This permanently damages the tissues and may lead to a heart attack or stroke."

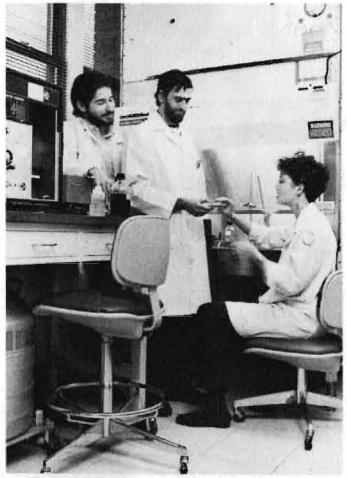
As the tape progresses, the patient learns that cholesterol is used by the body for making cell walls and hormones, that about three quarters of it is manufactured in the body, mainly in the liver, with the rest coming from cholesterol-containing



Jan L. Breslow

food, and that excess cholesterol is excreted in stools or stored in tissues and blood. He also learns that there are different kinds of cholesterol, and that the ratio of "bad" to "good" cholesterol – of LDL to HDL – is a much better predictor of risk for atherosclerosis than the total amount of cholesterol in the blood stream; the higher the LDL level, the greater the risk, the higher the HDL, the lower the risk.

Cholesterol by itself is insoluble. In order to travel in the blood it combines with proteins. This fat-protein package is called a lipoprotein. The terms LDL and HDL are abbreviations



Jonathan Smith, left, and Sheldon Feinstein in the tissue culture room where Ellen Johnson tends lipoprotein-producing liver cells.

Researchers Li-Shin Huang, left, and Hriday Das, with assistant Jeffrey Levine tracking apolipoprotein genes in the newly completed molecular biology laboratory.

for low-density lipoprotein and high-density lipoprotein, and refer to their weight and size. At the present time, precise measurements of LDL and HDL, and more refined measurements of the protein components of lipoproteins, can be done only in research centers like Rockefeller. These and other tests our patient will undergo, plus the counseling he and his doctor will receive from the hospital's researchers, nurses, and dietitians, will help him manage his condition in accordance with the best insights currently available to medical science. What he will give in return is some genes.

The laboratory of biochemical genetics and metabolism is under the leadership of Jan L. Breslow, a 41-year-old pediatrician and biochemist trained at Columbia University and Harvard Medical School. Before coming to Rockefeller in January 1984, he worked at Harvard and the Children's Hospital Medical Center, in Boston, for eleven years. Dr. Breslow is the latest of a number of recent additions to the Rockefeller faculty, invited to introduce new fields of investigation or new approaches in areas in which the University has traditionally been strong, like cholesterol research. Edward H. Ahrens, Jr., the subject of the first part of "Cholesterol-Watching" (*Research Profiles*, Fall 1984), has spent most of the last forty years tracking cholesterol through the body. Dr. Breslow is tracking the genes that control lipoprotein structure and function; in particular, those that code for the production of apolipoproteins, the proteins that surround the cholesterol core in lipoproteins.

ONE IN TEN BY FIFTY-FIVE

How much is atherosclerosis a matter of heredity and how much environment? Epidemiological studies have implicated a diet high in animal fats, as well as obesity, inactivity, smoking, and stress, among the factors that can contribute to atherosclerosis susceptibility. Although the recent trend toward a more prudent life-style appears to be having a beneficial effect, heart disease, most of it stemming from atherosclerosis, still accounts for half the deaths in the United States. Yet, as we all know, there are people who live high off the hog into their eighties and nineties (what Dr. Breslow calls the Winston Churchill syndrome), and there are seeming models of fitness whose vascular systems are timebombs. Tennis champion Arthur Ashe had a heart attack in his twenties and runner James Fixx died of one at fifty-two.

Genes and environment both play a role in the development of atherosclerosis, but, as Dr. Breslow points out, "in some people it's obvious that heredity dominates, for better or worse. Since we know that one in ten American men can expect to have a heart attack by the age of fifty-five, and of that group one in three will die on the way to the hospital, or within a month thereafter, we need to find the means to sort out those who are most susceptible and help them before they are stricken."

Dr. Breslow's interest in genetics and in diseases caused by inborn errors of metabolism was directed toward lipid research after medical school during a postdoctoral stint at the National Institutes of Health, where he worked in the laboratory of Donald Frederickson, who formulated what is now the standard system for classification of the hyperlipidemias. Invited back to Harvard in 1973 to establish his own laboratory at Children's Hospital, Dr. Breslow initiated the studies that this past September earned him the E. Mead Johnson Award, the major mid-career award of the American Academy of Pediatrics, for "outstanding work in the area of the genetic basis of the lipoprotein disorders." His research, reads the citation, "has far-reaching implications as we begin to realize that atherosclerotic heart disease has its beginnings in infancy and childhood."

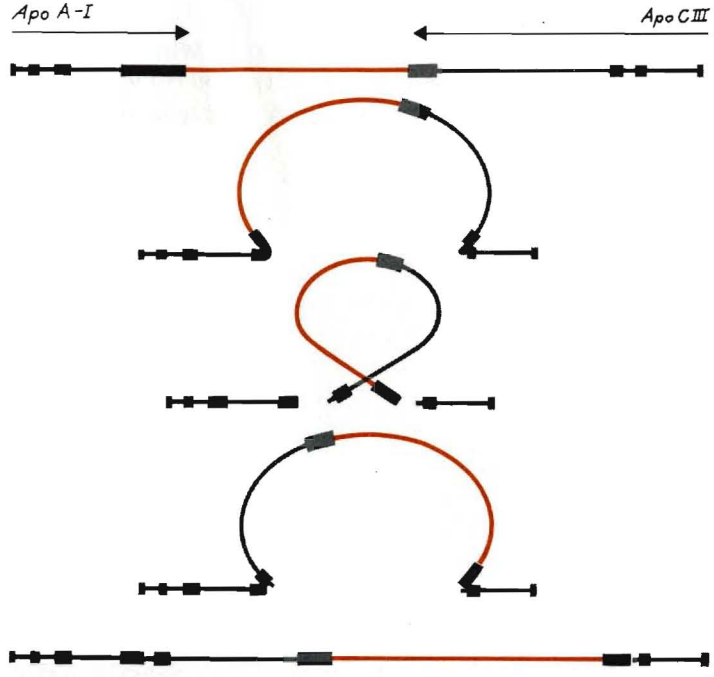
Dr. Breslow confers with fellow physicians, from left, Moshe Weintraub, Elliot Brinton, and Margo Denke.

APOLIPOPROTEINS

"There are several types of lipoprotein particles," Dr. Breslow explains. "They differ in the amount of cholesterol they carry and the types of apolipoproteins on their surface. LDL, which normally comprises about two thirds of the total cholesterol in the blood, is in charge of delivering cholesterol to cells. When a cell needs cholesterol, molecular receptors on its surface 'recognize' and bind with LDL apolipoproteins, an action that allows cholesterol to enter the cell. HDL acts as the cleanup squad that removes cholesterol from tissues and begins the process of transport to the liver for excretion in the bile."

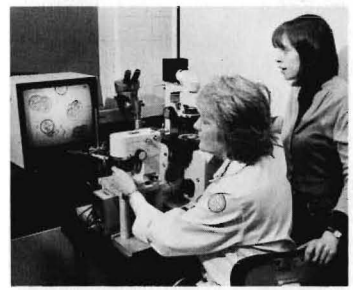
In normal metabolic functioning, cholesterol from the diet inhibits the synthesis of cholesterol in the liver so that the body can maintain a healthy balance. In some early animal experiments in Boston, Dr. Breslow discovered that a particle in the blood that came from the intestines after cholesterol feeding, and dramatically inhibited cholesterol synthesis, was very rich in one particular apolipoprotein, apo E. "Apo E had only recently been discovered," he says, "and we realized that if we were going to understand our observation, as well as other aspects of LDL and HDL regulation, we would have to make a systematic study of apolipoproteins, including their genes. Fortunately, there were a number of people in the research building where we worked, all investigating different diseases but all, like us, needing to incorporate the new genetic technologies that were just becoming available. So we pooled our resources and helped each other out. We learned as we went along."

Genes, which are made up of molecules of DNA, reside, in multicellular organisms, within the nucleus of each cell on structures called chromosomes. There are eight apolipoproteins, designated A-I, A-II, A-IV, B, CI, CII, CIII, and E; and eight genes that code for them. Dr. Breslow's laboratory was the first to develop methods for cloning – making copies of – the DNA of apolipoprotein genes. Mainly through his efforts, seven of the eight apolipoprotein genes have been isolated, characterized, and located on their specific chromosomes. The gene for apolipoprotein B, the major protein of LDL, has so far eluded capture. Its cloning was one of the first projects Dr. Breslow undertook with his new group at Rockefeller. "The preliminary results," he says, "look promising." Dr. Breslow was also the first researcher to use DNA technology to understand human disorders involving atherosclerosis.

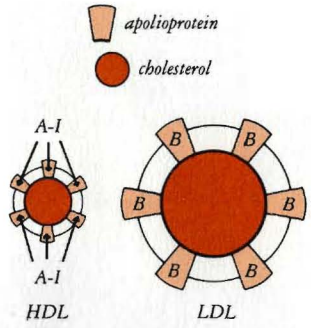


Rearranged apo A-I and apo CIII genes in patients with one form of severe premature atherosclerosis, as described on page 4.

"Working with apo E, we discovered that there are three relatively common forms of the gene, resulting in six possible patterns of the protein," Dr. Breslow relates. "We also discovered that patients with a condition called Type III hyperlipoproteinemia all have one particular pattern. This defective form actually occurs rather frequently in the general population, in about one in a hundred people, but the disease appears in only a small percentage of that group. This finding led us to suspect that an additional defect, in another gene, may be necessary for hyperlipidemia to develop, and we're looking for that second abnormality. We also think that the apo E or a closely linked gene may have other, more widespread effects on LDL cholesterol levels, which we're investigating.



Annemarie Walsh, seated, injects human apo A-I DNA into the nucleus of a fertilized mouse egg. She will then implant the egg in a female mouse. Some of the offspring in the resulting litter will carry the human gene making it possible to study the gene's activity. Supervising is Elizabeth Lacy, standing, Memorial Sloan-Kettering Cancer Center molecular biologist and one of the pioneers in the development of this technology.



Roberto Taramelli with laboratory helper Katie Tsang.

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"When we started to look at the apolipoproteins for HDL we again found an unexpected heterogeneity. Apo A-I is the major protein of HDL. As we learned, apo A-I is made in one form inside the cell and then undergoes a two-step modification before it becomes the mature plasma form. The second step occurs outside the cell in a process that seems to be unique to this apolipoprotein. In studying patients with Tangier disease, a rare condition in which HDL levels are extremely low, we observed that most of the apo A-I in their blood was in a precursor form. Now we're trying to find out whether this is due to a structural defect or a defect in the converting activity.

"A particularly tantalizing finding came out of another study, of a family referred to us in which there was an unusually high incidence of severe premature atherosclerosis, and in which two sisters had had heart attacks in their twenties. These individuals had extremely low HDL levels and lacked apo A-I and another apolipoprotein, apo CIII, in their plasma. From our previous work we knew that the genes for both these proteins are very close to one another on the same chromosome. When we looked at DNA samples from the sisters, we discovered that part of the apo CIII gene had left its proper position and become inserted into the apo A-I gene. This DNA rearrangement had inactivited both the apo A-I and the apo CIII genes. It's pretty awesome to think of a single gene abnormality that can accelerate the age for a heart attack by fifty years.

"About five percent of the population has half-normal HDL levels. Having demonstrated in a prototype situation that a lesion in the apo A-I gene was the cause, we're very eager to find out if that or similar apo A-I gene lesions might be an explanation for the general phenomenon."

A LABORATORY IS BORN

It's been a hectic year for Dr. Breslow, recruiting a research team, house-hunting for his family, and overseeing the monumental task of transforming the seventh floor of the Rockefeller Hospital to accommodate molecular technology and his own six-foot-four-inch frame. By next summer, when all the members of the laboratory have finally arrived from around the country and the world, they will number about twenty-five, including support staff.

"They're a terrific group," says Dr. Breslow, "Hriday Das, a molecular biologist from Yale, who's working on the organization of human apolipoprotein genes, has recently discovered that the gene for apo CI resides very close to the apo E gene. Sheldon Feinstein, from Yale and the Weizmann Institute, is looking at the expression of the apo A-I gene. Roberto Taramelli, who came from Italy via the Medical Research Council, in England, is cloning apolipoprotein genes from patients with lipoprotein disorders to determine the nature of the DNA defect. Jonathan Smith, a new Ph.D. from Harvard, is studying apo E expression in human liver-cell cultures. Li-Shin Huang is an immunologist who joined us from the University of Delaware. She's applying immunological techniques to clone the gene for apo B. Annemarie Walsh, a nucleic acid chemist, trained at New York University and worked at the University of Michigan. She's going to see if by introducing the human apo A-I gene into the germline of an atherosclerosis-susceptible mouse she can turn it into a resistant one. This system, which she's learning from colleagues across the street at Memorial Sloan-Kettering Cancer Center, will also allow us to study the function of other apolipoproteins.

"There are three M.D.s in the lab in addition to myself. Dr. Denke, who's just finished her clinical residency at Brigham and Women's Hospital, is concerned with the nutritional aspects of atherosclerosis. Besides being the star of our orientation tapes, she's involved in studies of LDL metabolism and the development of an immunoassay for apo B. Elliot Brinton is an endocrinologist. He came from a three-year fellowship in Seattle during which he defined cellular receptors for HDL. He's now looking at HDL turnover in patients with coronary artery disease. Moshe Weintraub is a cardiologist from Israel who has been in clinical practice for a number of years and is interested in learning research techniques. He's applying the methods we established in Boston for identifying the six types of apo E to examining their role in the metabolism of dietary fat."

The patients who come to Dr. Breslow's clinic may not know much about genetics or the apolipoprotein alphabet. "What they do know," he says, "is that we take a lot of blood from them." They also know that within the DNA in those blood cells may lie the key to their children's future health.