GENETICS

Newfound Gene Holds Key to Cell's Cholesterol Traffic

At age 4, Marcia Parseghian was the most coordinated student in her dance class. Now 8, "she's having a real hard time keeping up with the other girls," says her mother, Cindy Parseghian. "She stumbles a lot." Her stumbling is just one sign that Marcia's nervous system is decaying. Like an older brother who died 4 months ago from a seizure and a sister who is 2 years younger, Marcia suffers from a rare genetic disorder called Niemann-Pick type C disease. Because the cells of Niemann-Pick patients cannot process cholesterol, they become glutted with the fatlike molecule. Nerve cells are the first to die, causing problems in seeing, walking, hearing, and swallowing. Like their brother, the two Parseghian girls are not expected to reach adulthood.

When the parents learned in 1994 that three of their four children were affected by this disease, "we were devastated," Cindy Parseghian recalls—but not defeated. Together with Ara Parseghian, the grandfather and a well-known college football coach, they formed a foundation that has raised $6 million to support research on this obscure disease. That support has begun to pay off.

On page 228, a team of some three dozen investigators—funded in part by the Ara Parseghian Medical Research Foundation and another parents' organization called the National Niemann-Pick Disease Foundation—report that they have tracked down the gene that causes the disease. The discovery "is a very big step forward," says Yvonne Lange, a cell biologist at Rush-Presbyterian-St. Luke's Medical Center in Chicago. Already, the gene's nucleotide sequence is yielding some early clues to the function of its protein, says Peter Pentchev, a cell biologist at the National Institute of Neurological Disorders and Stroke (NINDS) in Bethesda, Maryland, who is one of the leaders of the research team. The Niemann-Pick protein, called NPC1, seems to sense a cell's level of cholesterol and help shuttle it from one part of the cell to another.

A second result, reported on page 232, should help researchers test those ideas. Molecular biologist William Pavan of the National Human Genome Research Institute (NHGRI) in Bethesda, Maryland, and his colleagues have found that an identical gene is at fault in a mouse model of the disease. The availability of a precise mouse model should open the way for detailed study of how the NPC1 protein causes disease when it is disabled and could lead to the development of a treatment.

The Niemann-Pick protein should also shed some light on how normal cells manage cholesterol, which is a component of cell membranes, an essential building block for steroid hormones—and an ingredient of the artery-clogging deposits of atherosclerosis. "[The gene] is going to be relevant to all problems in cholesterol homeostasis, especially atherosclerosis," Lange predicts. "[These] are important and elegant studies," agrees Joseph Goldstein of the University of Texas Southwestern Medical Center in Dallas who, with his colleague Michael Brown, won a Nobel Prize for studies of cholesterol regulation.

PARSEGHIAN FOUNDATION

CARSTEA *ET AL.*

Cholesterol's curse. Three of the four Parseghian children, pictured with their parents and grandfather, have Niemann-Pick type C disease, in which cholesterol (blue) builds up in cells.

Pentchev has been on the trail of the Niemann-Pick gene for some 20 years, ever since he began studying a mutant mouse strain that mimicked the human symptoms. Five years ago, Eugene Carstea, now at St. Mary's Hospital and Medical Center in Grand Junction, Colorado, joined Pentchev's lab, and the team's interest escalated into a full-scale gene hunt.

With the help of Danilo Tagle and other colleagues at NHGRI, Carstea and NINDS's Jill Morris first narrowed their search to a 1.5-million-base-pair region along chromosome 18 by linkage analysis in families with the disease, including a large Bedouin family from Israel. To close in on the gene, Jessie Gu and Melissa Rosenfeld of the NHGRI obtained yeast artificial chromosomes (YACs) that contained different pieces of that entire chromosome region. They added each YAC to a set of cultured cells that had the Niemann-Pick defect and looked for cells that could then process cholesterol correctly. Those cells must have received a YAC containing a normal copy of the gene.

The overlap between the human DNA in the YAC and the region defined by linkage analyses was 300 kilobases. The researchers further subdivided this region and picked out the protein-coding regions within each piece. By comparing those coding sequences to fragments of unknown genes archived in the public database called GenBank, they were able to piece together and sequence a complete gene. That gene turned out to be the one mutated in cells of patients with the disease, confirming that the search was over.

This hunt gave a boost to another quest that had been under way for 2 years: finding the gene defect in mutant mice with the same symptoms as human sufferers. Already, several research teams had shown that the defective gene was on mouse chromosome 18—the same chromosome as in people. By combining linkage data from mouse-breeding studies with information about the gene's location in humans, Pavan, NHGRI's Stacie Loftus, and their colleagues narrowed the candidates to two possible genes.

Of the two, one was much less active—as indicated by the amount of its messenger RNA—in liver and brain cells of mice with Niemann-Pick disease than in those cells of normal mice, suggesting that it was the defective gene. When Pavan, Tagle, and their colleagues compared the suspect gene's sequence to that of the human *NPC1*, they confirmed that the two are identical. "The mouse paper is a terrific confirmation of the human gene defect," concludes Laura Liscum, a cell biologist at Tufts School of Medicine in Boston.

Because the mouse and human genes are so similar, researchers will have an easier time sorting out the role of the NPC1 protein, which "will be central to understanding the process of how cells deal with cholesterol," says Goldstein. In particular, the protein could help fill out a picture that Goldstein and Brown began developing 25 years ago. Their studies of another rare disease, familial hypercholesterolemia, helped them work out how the body maintains the proper amounts of cholesterol in cells. They showed that cholesterol from the bloodstream binds to a docking site called the LDL receptor on the cell surface. The receptor is

then drawn into the cell's interior and the cholesterol freed in a compartmentlike cell organelle called a lysosome.

Brown and Goldstein found that cholesterol ultimately gets incorporated into the cell membrane or repackaged for storage or breakdown. They and others also showed that when a cell is glutted with cholesterol, feedback mechanisms kick into action: The cell stops making LDL receptors and shuts down its own cholesterol-synthesizing processes.

Even though Brown and Goldstein worked out much of the beginning and end of cholesterol's journey, "how free cholesterol moves out of the lysosome and into other parts of the cell [remained] a black box," explains George Rothblat, a cell biologist at the MCP-Hahnemann School of Medicine in Philadelphia. "This is where the defect is in Niemann-Pick disease." Somehow, the defective protein hampers the cholesterol's movement through the cells and the feedback mechanisms that control its level. "It's a protein that's screaming for attention," says Liscum.

Already, an examination of the NPC1 protein has revealed a stretch of amino acids where it resembles other proteins known to interact with cholesterol to regulate its level. Another section of the NPC1 protein looks as if it may somehow interact with the lysosome. In between are 16 regions with sequences that indicate, says Carstea, that "this [protein] is locked in a membrane" somewhere inside the cell.

Making sense of those clues will take more study, but in the meantime, the gene may lead to other trafficking molecules. "There could be a whole family of these proteins," says Goldstein. For example, a very small subset of people with Niemann-Pick type C disease have a different genetic defect, located on another chromosome. It affects a protein that seems to function as a trafficking molecule, much like the NPC1 protein.

The identification of the *NPC1* gene may also be a step toward developing a treatment. "We're hopeful that having the gene will let us get at a therapy faster," says Liscum, who hopes to use cell cultures and mice to screen for drugs that might slow cholesterol buildup in Niemann-Pick children. She cautions, however, that "it's going to be a long time coming in having an impact on kids."

While Cindy Parseghian realizes that the new gene find may not help her daughters, she thinks it may help other family members, by leading to a way to test for the mutant gene in those who do not have symptoms, including her one healthy son. In the meantime, she and her husband make the best of the time they have with their daughters. "We can look at our children as if they were dying, or we can look at them as though they are living," she points out. "They look at life as if they are living, and that's what we try to do."

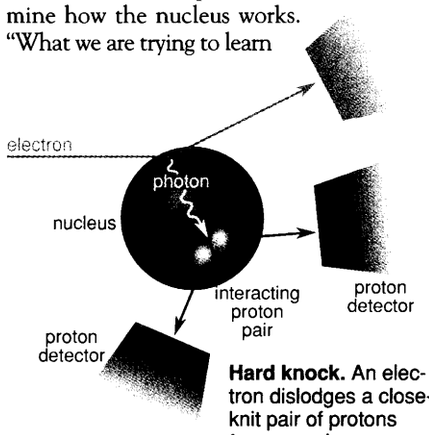
–Elizabeth Pennisi

NUCLEAR PHYSICS

Accelerator Probes the Private Lives of Nucleons

Census data can tell us much about how the average person lives, but they inevitably miss the interplay between individuals that ultimately makes our societies tick. Nuclear physicists face similar difficulties in their attempts to fathom the crowds of particles that populate the atomic nucleus. Nuclei contain as many as a few hundred protons and neutrons, collectively known as nucleons, as well as a sea of other particles that bind the nucleons together. The interaction of so many particles is immensely complicated, so physicists have had to content themselves with models that average out nucleon behavior.

Now, however, a team at NIKHEF, the Netherlands's National Institute for Nuclear and High-Energy Physics in Amsterdam, has been able to coax pairs of protons out of the nucleus just at the moment when their interactions are at their most intense, revealing the intimate relationships that determine how the nucleus works. "What we are trying to learn is how the fact that the two nucleons are bound in the nucleus influences their mutual interaction," says NIKHEF's Gerco Onderwater.



Hard knock. An electron dislodges a close-knit pair of protons from a nucleus.

Other nuclear physicists are thrilled to have this intimate glimpse of nucleons, which the Dutch team offers in the 30 June issue of *Physical Review Letters*. "The NIKHEF experiment demonstrates that it will be possible to learn about how protons interact with each other *inside* the nucleus," says Wim Dickhoff of Washington University in St. Louis. Adds Carlotta Giusti of the University of Pavia in Italy: "This is a fundamental milestone in the understanding of nuclear structure."

Each nucleon is made up of three quarks bound by the strong nuclear force. This force also holds nucleons together as they exchange force-carrying particles called mesons. However, calculating the details of how the hundreds of nucleons and mesons interact is impossibly complex, so physicists fall back on statistics. They define a single, averaged force field, or mean field, which describes how a nucleon interacts with its averaged neighbors. Mean-field models "are able to reconstruct and explain a lot of details with enormous accuracy in nuclear structure," says Onderwater. "Until now, these mean-field approximations worked fine."

But experiments are beginning to reveal cracks in these models. According to Onderwater, experiments using electrons to knock out single protons only seem to see about 65% of the proton population in the nucleus. Moreover, the momentum of many of the knocked-out protons is unacceptably large according to mean-field models, which predict that such boisterous nucleons should spontaneously fly out of the nucleus. "A proton in the nucleus spends only a fraction—albeit a sizable one—of its time in this average field," says Dickhoff. "In fact, because it interacts strongly and collides with other nucleons, it spends about 35% of its time doing 'other' things."

Theorists are now attempting to patch up the mean-field model by incorporating short-range correlations (SRCs) between nucleons, describing how nucleons influence only their nearest neighbors and not the nucleus as a whole. The main short-range effect is a switch from attractive to repulsive forces when nucleons are very close together. The repulsion ultimately keeps nucleons apart and prevents nuclei from collapsing to a fraction of their normal size.

In this picture, nucleons consist of a repulsive quark core surrounded by a cloud of attracting mesons. "The high energies and momenta arise from violent collisions in which the quark cores touch each other," says Derek Branford of the University of Edinburgh in the United Kingdom. The NIKHEF experiment, he says, sets out to test this image. "It is designed to catch two protons at the time they are so close that the quark cores are touching, and knock them out of the nucleus."

To do this, the NIKHEF team modified its Amsterdam Pulse Stretcher electron accelerator, transforming its beam of brief electron pulses into a more or less continuous beam, to increase the chances of catching close interactions. The beam, which has an energy of 584 mega–electron volts, is trained on a water target surrounded by three detectors. One captures electrons that bounce