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These girls have DEMENTIA

WILL THEIR BRAINS REVEAL CLUES THAT CAN SAVE MILLIONS OF ALZHEIMER'S PATIENTS?

THE TRUTH ABOUT ESTROGEN

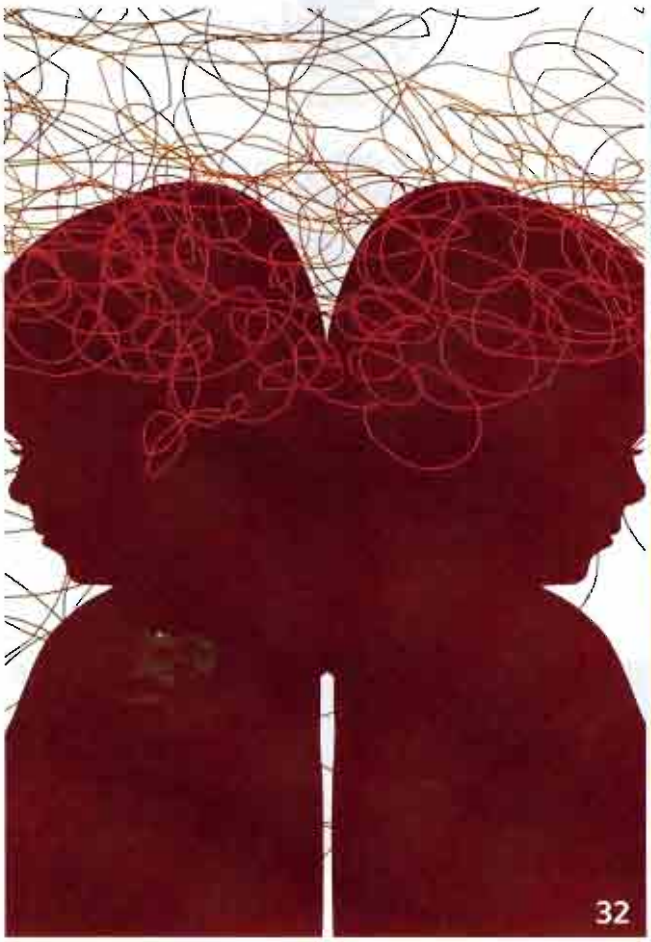
BEST PLACES TO WORK: ACADEMIA

HOW SCIENCE LIBRARIANS CAN SAVE YOU HOURS OF WORK

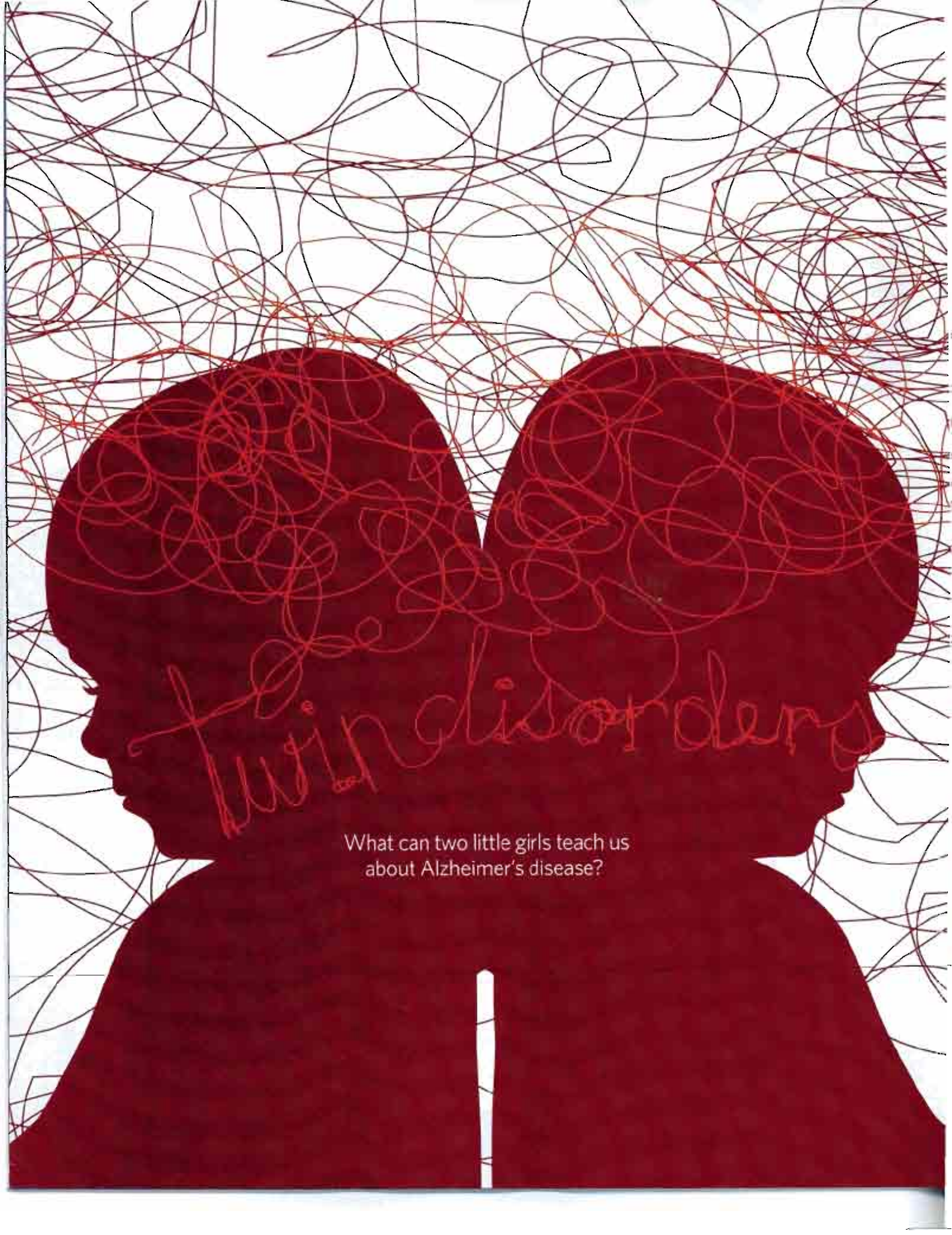
PLUS:
NIH'S GISELA STORZ

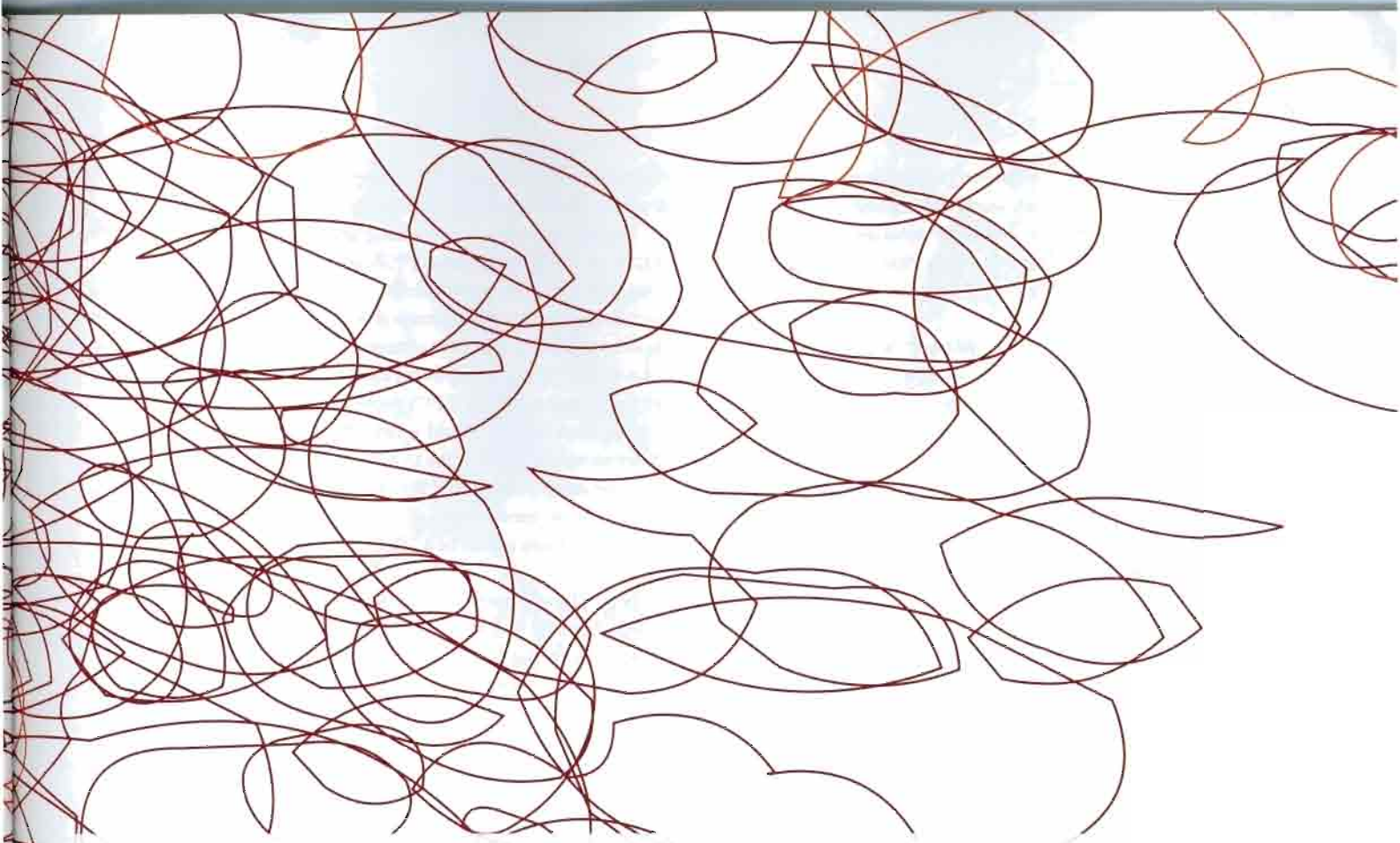
CONTENTS

November 2008



FEATURES


twin disorders
What can two little girls teach us
about Alzheimer's disease?



By Alison McCook

When you meet identical four-year old twins Addi and Cassi Hempel, you might notice something about the way they walk. They used to run around like other toddlers, but now they are more wobbly, more uncertain, and walk with their legs somewhat wide apart, as if aboard a boat. They can sway in any direction, losing their balance. They fall more often than they should.

They will notice you, and smile. They don't say words but they talk, a rhythmic, nonsensical babble from which a crystal-clear sound occasionally escapes: "ice cream," "paddycake," "four." Their heads have a slight bobble, and they sometimes can't angle their eyes downward, so they fall again.

Unlike most children, who get better at things with time, Addi and Cassi's gait will get worse, and they'll reach more for railings and furniture for support. They'll fall more, adding to the bruises that already dot their elbows and knees. The few steps in their parents' newly renovated house will become impossible; when walking gets too difficult, they'll use a wheelchair. They're not potty-trained, and likely never will be.

They will stop saying words, and may stop speaking altogether. Soon, they'll start to forget things they once remembered; like which bed is whose in the room they share, or who their parents are. They may start to have seizures. As their condition worsens, their swallowing will deteriorate, and their parents may place them on feeding tubes. In several years, they will likely die – first one, then the other.

Addi and Cassi have a rare genetic disease known as Niemann-Pick Type C (NPC), which affects cholesterol trafficking inside cells. There is neither a treatment nor a cure.

NPC is exceedingly rare – its estimated prevalence is 1 in 150,000 – but it has captured the interest of a disproportionate number of scientists. Addi and Cassi were diagnosed with NPC in October, 2007, and one month later, their parents, Chris and Hugh, flew to the National Institutes of Health to meet with the senior advisor to the director for translational research at the National Human Genome Research Institute (NHGRI), Christopher Austin. As a result of that meeting, Austin, a former Merck researcher, is now screening 3,000 approved drugs, looking for something that corrects the disease phenotype in the twins' cells.

Chris Hempel, co-founder of a technology PR agency, didn't stop there. Within months, she, Hugh, and other parents of NPC kids helped assemble an unprecedented collaboration between NPC researchers, gathered samples for a longitudinal study to look for NPC biomarkers, assisted one of the world's leading stem cell researchers in getting the background he needed to build an NPC neuron using human embryonic stem cells, and threw a fundraiser that netted $500,000 for NPC research. They plan to use part of the funds to pay a scientist to create a mouse model of NPC with Addi and Cassi's specific mutations.

One reason scientists have been eager to investigate such a rare disease is the fact that they can enjoy funding from the Ara Parseghian Medical Research Foundation, named after a football coach whose grandchildren had NPC (see related story, p.92). But there is another, important reason. NPC shares key features with ▸

another deadly disorder that currently affects as many as 5 million Americans, and millions more worldwide: Alzheimer's disease.

NPC "is of enormous basic science interest," says Lawrence Goldstein, who uses stem cells to study Alzheimer's at the University of California, San Diego.

When Addi and Cassi were two, they both developed a severe case of mononucleosis, at which time their doctor noticed they had enlarged spleens. Over the next year and a half, doctors continued to test the girls' blood and urine, searching for an explanation for their over-sized spleens. Nothing. Then, the girls, who were running around like healthy, energetic toddlers, began to have trouble balancing. The parents pushed for more testing, until finally, nearly two years after the first symptoms, a doctor ordered skin biopsies to check for NPC.

It confirmed the Hempels' worst fears. Inside Addi and Cassi's cells was a mutation on each *NPC1* gene, located on chromosome 18. The protein that *NPC1* encodes helps transport lipids from the late endosomes to the plasma membrane and ER. A mutation in the gene disrupts its activity, causing cholesterol and other lipids to build up in cells. It's not exactly clear why cells affected by NPC die, but they do – often neurons that control eye movement and Purkinje cells, which explains why symptoms of NPC include difficulty with walking and coordination. In cells of peripheral tissues, the primary lipid that accumulates is unesterified cholesterol, and the diagnostic test, a skin biopsy, checks if fibroblasts are accumulating cholesterol. Genotyping is not always an option, because there are many different mutations that can disrupt *NPC1*.

NPC is an autosomal recessive disease, but most patients are compound heterozygous. Scientists have since identified Chris' mutation on her NPC gene, but haven't yet found Hugh's. (Despite the fact that scientists can often pinpoint the specific mutations behind NPC, gene therapy is not an attractive option, because NPC1 is a transmembrane protein that is not released from cells, so gene therapy would have to correct every cell to have an effect.)

Christopher Austin first became interested in NPC 20 years ago as a clinical neurologist treating children with the disease. A career later, he found himself sitting face to face with Chris and Hugh Hempel, who were still reeling from their daughters' diagnosis only weeks earlier. They asked him if he could test Addi and Cassi's cells against a slew of approved drugs to look for something that might slow their disease progress, to skip over years of lab research their girls couldn't wait for. Austin thought: Yes.

So Austin started a new experiment, in which he began screening cells from NPC kids – including Addi and Cassi – against 3,000 approved drugs. The experiment, funded by the Hempels and the Ara Parseghian Foundation, uses the diagnostic test for NPC to check if NPC fibroblasts exposed to a drug have reverted to a normal phenotype. The beginning stages will wrap up this fall, Austin predicts, and there are already early results. "There are definitely compounds which make the diagnostic phenotype go away. But there are so many caveats to that," he says. His lab is running an uncountable number of tests, since every child's cells is screened against different concentrations of thousands of drugs, so there are likely to be some false positives.

Simultaneously, he is working with two other NPC researchers, Steve Walkley at Albert Einstein College of Medicine and, primarily, Dan Ory at Washington University, to screen cell lines engineered to express a common NPC genotype against different concentrations of 400,000 compounds. In this case, the researchers will check if a compound causes diseased cells to produce normal versions of the NPC1 protein.

But then what? Would a drug that comes up as a match in either screen be safe to give to NPC kids? "The $64,000 question is: Is the assay predictive? We just don't know that," says Austin. "It doesn't get more translational than this. We're talking about going right from a dish to a kid."

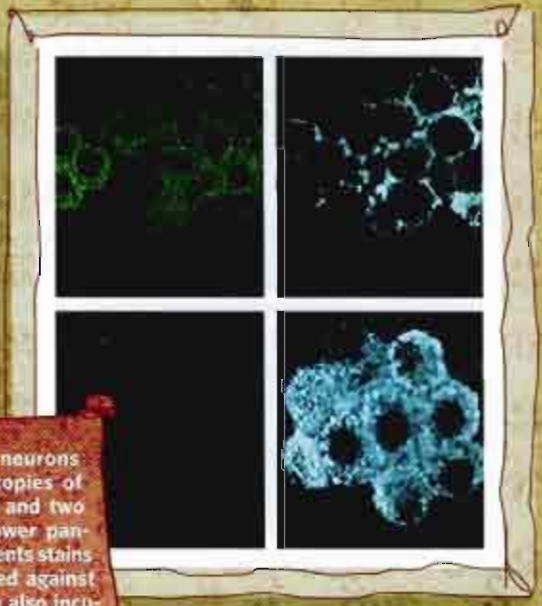
The connection to Alzheimer's Disease is initially what attracted Lawrence Goldstein to study NPC, which is even colloquially known as "Childhood Alzheimer's." "There are some hints – that may or may not turn out to be correct – that there are some similarities in some of the biochemical pathways" between NPC and Alzheimer's, Goldstein notes.

Soon, the girls will likely forget things they once remembered; like which bed is whose in the room they share, or *who their parents are.*

Addi (left) and Cassi (right)

Chris Hempel with the girls

Hugh Hempel with the girls



Mouse sympathetic neurons with two functional copies of NPC1 (upper panels) and two mutated versions (lower panels). The green represents stains from antibodies raised against NPC1. The cells were also incubated with the cholesterol binding compound, filipin (blue). In healthy cells, cholesterol is largely on the cell surface; in diseased cells, cholesterol is more widely distributed.

Both disorders have obvious links to cholesterol – in NPC, cholesterol is not trafficked properly around the cell. High cholesterol appears to increase the risk of Alzheimer's, and apoE4, a variant of the protein apolipoprotein E, influences the development of both NPC and Alzheimer's. Cholesterol also accumulates in the cells of people with Alzheimer's, although that is not as prominent an aspect of the Alzheimer's pathology as other features.

Alzheimer's disease, says Thomas Ohm of Charité – University Medicine Berlin, in an E-mail.

Interestingly, NPC mouse models do not form tangles, and learning why "seems to be crucial in our further understanding of tangle formation," Ohm predicts. Based on published data and his group's unpublished work, they know that the mouse tau protein can form tangles in vitro, but in vivo, tau is not phosphorylated the way it is

> "We are finding more and more reasons to draw similarities between lysosomal storage diseases like NPC in general and Alzheimer's."
>
> – RALPH NIXON

Strikingly, one of the main hallmarks of cells affected by Alzheimer's – neurofibrillary tangles, made up of hyperphosphorylated forms of the protein tau – are present in "virtually identical" form in NPC cells, says Ralph Nixon, director of the Center for Dementia Research at the Nathan Kline Institute in Orangeburg, New York. NPC tangles are intraneuronal as long as the neuron lives. Then they end up as "ghost" tangles, as in Alzheimer's, and no tangles form. "Maybe this [Alzheimer's]-like phosphorylation state is a prerequisite for tangle formation, and mice harbor factors which hinder this kind of phosphorylation." If researchers can identify those factors, "we may have found a new therapeutic strategy for AD and human NPC," says Ohm.

Recent findings also show that some NPC neurons develop "primitive plaque-like structures," signs of the other hallmark of Alzheimer's, brain plaques. A recent genome-wide expression analysis of fibroblasts with mutated *NPC1* revealed higher expression levels of genes that help generate amyloid.[1] Neurons affected by both diseases experience dramatic accumulations of autophagic vacuoles and lysosomes, and cells affected by both disorders have enlarged endosomes, a feature seen in only a small number of disorders, says Nixon.

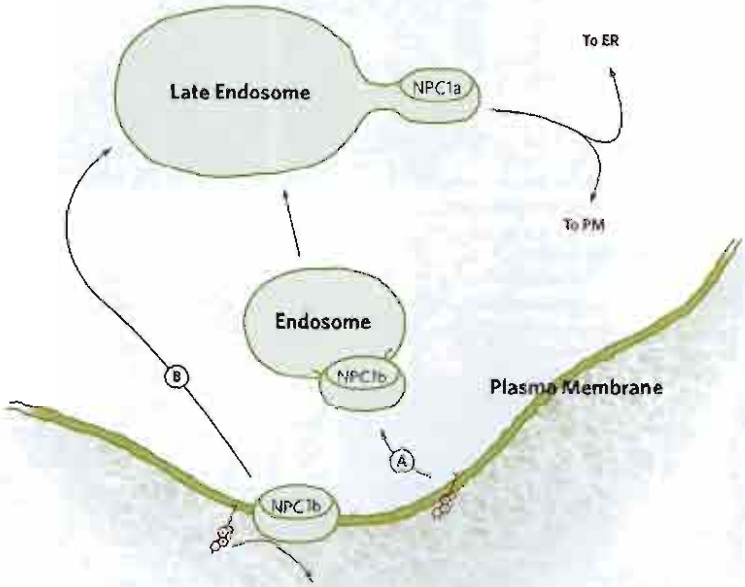
The similarities are strong enough that some researchers study NPC because of its link to Alzheimer's. "It is certainly one of the reasons we chose to get into Niemann Pick ourselves," Nixon says. "We are finding more and more reasons to draw similarities between lysosomal storage diseases [like NPC] in general and Alzheimer's."

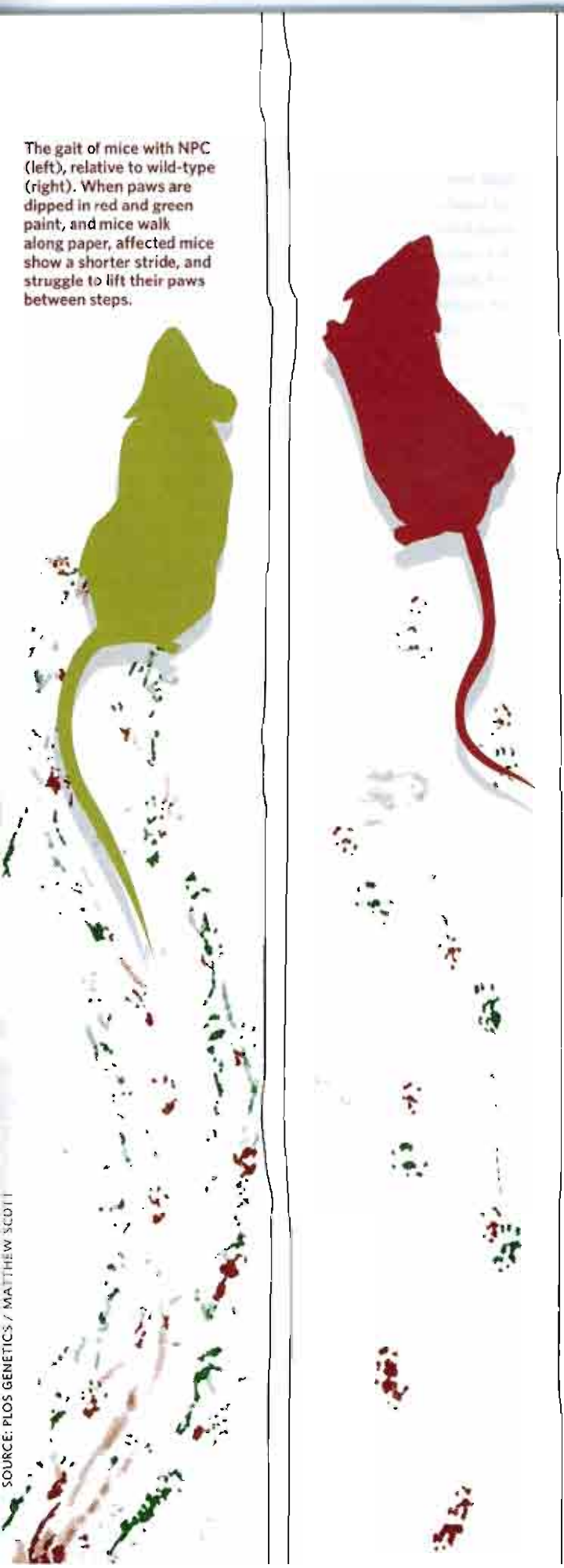
When Goldstein chose to follow suit, however, he realized he knew little about the rare disease. In jumped Chris Hempel, who helped arrange one-on-one and conference phone calls between Goldstein and the NPC scientists she knows to educate him about its molecular underpinnings. "Parents were very helpful in facilitating that and moving me into this new area," Goldstein says.

He is now building an NPC neuron using human embryonic stem cell lines, which no longer express the NPC gene. "We're differentiating them now." The goal, Goldstein says, is to use the neurons to screen drug candidates. The technology doesn't exist to perform a high throughput screen using neurons, but he says that "it's not outside the realm of possibility" to use NPC neurons to test compounds flagged during HTP screens of non-neuronal cells. "We don't know how useful [an NPC neuron] will be," he says. "Personally, I think it will be incredibly useful."

Role of NPC1 in healthy cells

The initial step of cholesterol absorption is facilitated by NPC1b, either at the plasma membrane (B) or in an intracellular compartment such as an early endosome (A). Once at the late endosome, NPC1a regulates cholesterol's transport to the endoplasmic reticulum or the plasma membrane.





The gait of mice with NPC (left), relative to wild-type (right). When paws are dipped in red and green paint, and mice walk along paper, affected mice show a shorter stride, and struggle to lift their paws between steps.

SOURCE: PLOS GENETICS / MATTHEW SCOTT

In a kitchen with large windows that sometimes cloud over with smoke from nearby California wildfires, an island contains a tray piled with the plastic syringes, droppers, powders, and capsules the Hempels are using to try to arrest the girls' disease. The high-ceilinged kitchen is the center of life in the house, where Chris keeps her computer so she can check E-mail and correspond with scientists while staying connected to her household. This includes her mom and dad, who divorced decades ago but now sleep in separate rooms at the Hempels' to help out with the kids.

Sitting at the kitchen island, Chris reaches for the tray, picks up a white, plastic sheet of pills, and points to one capsule: "Each of these is roughly $200," she says, adding up to $160,000 per year for both girls to take the drug (thankfully, it's covered by insurance). The drug is the only prescription that comes with some evidence it may help NPC: Zavesca (miglustat), an inhibitor of glycosphingolipid synthesis, normally used to treat another lysosomal storage disorder, Gaucher disease.[2]

Chris's palms, knuckles and fingers are frequently stained orange from the curry spice curcumin. Her decision to try curcumin as a treatment for her kids came out of experiments by University of Oxford researcher Frances Platt, who Chris found through the Internet. Platt has been investigating NPC for years, and suspects that the dysfunctional NPC1 protein disrupts the regulation of calcium in the cell, and pharmacological agents that cause calcium to increase in the cytoplasm could correct this disruption. Platt and her colleagues found that curcumin could elevate cytosolic calcium; she tested it in NPC mice, and found it improved their disease. But Hempel didn't know this when she contacted Platt – she simply saw Platt was working on curcumin and NPC, and tried "begging for information" about whether curcumin could help her girls. Platt cautioned her that her research was preliminary, performed in mice, etc. But, yes, Platt conceded, maybe curcumin could help her girls.

Currently, the girls swallow twice-daily doses of powdered curcumin, which stains anything they put their mouths on, such as the nose of Cassi's favorite stuffed dog. The daily amount is 3,000 mg of curcumin and other ingredients added by the company that manufactures the supplement. It's hard to tell if anything is slowing the girls' inevitable deterioration, but there are hopeful signs: Months after her diagnosis, Cassi didn't even make eye contact, but on a sunny afternoon in July, she smiles at other people, even laughing when her sister farts. "I know how well they're walking because of the bruises," Chris Hempel says, and she believes the girls are doing better. Early evidence also suggests curcumin could help treat Alzheimer's disease.[3]

Platt is part of a group of researchers known as SOAR-NPC, or Support of Accelerated Research for Niemann-Pick disease type C. SOAR-NPC is managed by Collabrx, a new "virtual" biotech that facilitates collaborations between researchers. The purpose, says Smruti Vidwans, a molecular biologist-turned-drug development strategist who oversees the NPC group at Collabrx, is to find therapies faster by getting scientists to ▸

develop a targeted strategy for doing so, share ideas and data, and making sure no one doubles another's efforts. In exchange, researchers receive professional consulting and management services (such as Vidwans and Collabrx's software platform, which lets them share data and ideas remotely). They also receive funding from the Hide & Seek Foundation (which funds lysosomal diseases) and, primarily, Dana's Angels Research Trust, an NPC foundation.

Besides regular conference calls with each other and Vidwans, scientists have to be up for "real time peer review" in which they are constantly asking each other if they're doing the right studies, looking for the right outcomes, and so forth, says Jonathan Jacoby, COO of Collabrx, adding he is in "daily" contact with NPC scientists. "You discuss things as they happen within the group of researchers involved, which is actually quite different" from the normal process, says Platt.

The Hempels, who are using some of the money they raised to fund Collabrx scientists, met at software company Netscape, a company known for publicly releasing the code of its software, and they were initially shocked to learn that scientists traditionally restrict access to their data until it's published, or meet their colleagues only at yearly conferences. Scientists "don't act like Internet startups," says Chris Hempel. "They're like, 'We'll call you in two weeks.' And we're like: No, you need to call us tomorrow!" She says she hopes the SOAR-NPC system becomes a model for other diseases.

Last year, SOAR scientists identified more than 10 therapies to test in mice, and as of this summer, nearly all were being tested. They are already starting to think about the right combination therapy, Vidwans says. "This is probably something that wouldn't have happened without SOAR."

Twice now, Addi and Cassi Hempel have flown across the country to Bethesda, Md., to meet with Forbes (Denny) Porter, at the National Institute of Child Health and Human Development. They are part of his longitudinal trial of NPC, designed to define biomarkers researchers could use as part of a clinical trial and diagnostic test for NPC.

Chris Hempel also helped with this project. Once her kids were enrolled in Porter's trial, she asked him if there was anything she could do. Actually, there was: "I'm at the NIH, so we don't see many control kids," Porter told her. The study needed to compare blood and urine samples from NPC kids to those from healthy kids, and most kids who come through the NIH have some sort of a disease. She and another NPC parent immediately got to work on a urine and blood drive, and once Porter obtained approval from his IRB, "it took a couple of weeks" for the parents to send between 30 and 40 blood samples, and the same number of urine samples, he says. "It saved months of work and difficulty," Porter says.

Ideally, any discovery that comes from the labs of Porter, Austin, Goldstein, or any of the Collabrx researchers would benefit more than the handful of NPC patients. "Our fondest hope" is that insights into how NPC works will yield insights into other disorders of cholesterol metabolism, such as Alzheimer's, and even cholesterol disorders in general, says Austin. (Nobel laureates Michael Brown and Joseph Goldstein studied severe hereditary familial hypercholesterolemia and discovered LDL receptors, which led to new ways of treating atherosclerosis.) "I think it's perfectly possible that what we discover [in NPC] will be useful to Alzheimer's disease and other neurodegenerative diseases," says Austin. "There's a long history of rare diseases shedding light on more common ones. This wouldn't be the first."

> NPC "is of **enormous** basic science interest."
>
> – LAWRENCE GOLDSTEIN

But for the most part, the only patients on Chris and Hugh Hempel's minds are Addi and Cassi. Standing in the kitchen making a quesadilla for himself and Chris, Hugh raises the idea of feeding tubes, for when the girls have trouble swallowing and could aspirate small particles of food or liquid into their lungs, putting them at risk of pneumonia. Parents obviously have to make the decision of whether or not to do it, but the responsibility of it is daunting, he says. "We're not going to get there." Chris asserts, resisting the possibility of her girls getting that sick.

Later on that day, Chris sits in front of her computer and quietly watches a video clip of a little boy with an advanced form of NPC, who doesn't move and makes no eye contact with his mother, who is talking to him off-camera. "It's heartbreaking," Chris says as the movie ends. It's impossible to imagine that her kids, who can still walk and talk, will one day be bedridden too. "That thought of kids losing their mind – I don't know," she says. "It's got to be one of the cruelest diseases on the planet for kids. And for parents, if they can't remember who you are." ■

Have a comment? E-mail us at mailto:the-scientist.com

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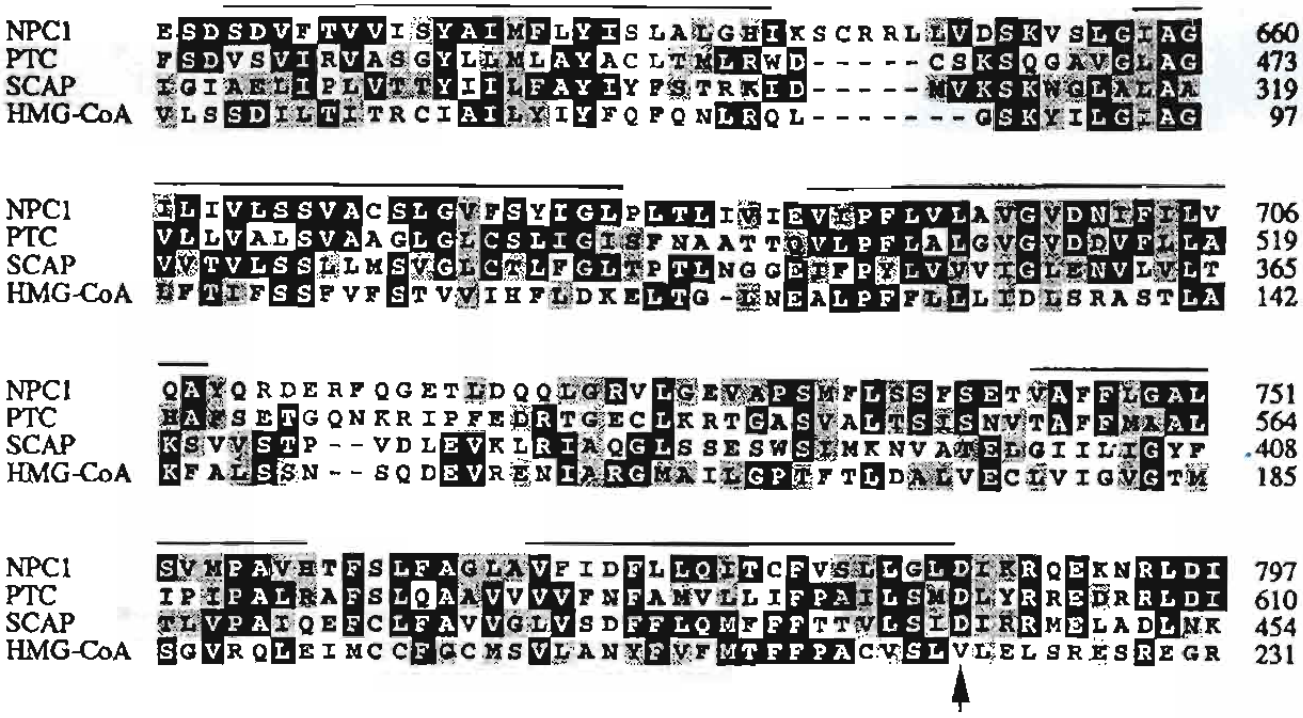
By Jennifer Evans

FOUNDATIONS

Cholesterol and NPC1, circa 1997



NPC1's amino acid sequence homology to PATCHED, human HMG-CoA reductase and SCAP.

In the 1990s, the Ara Parseghian Foundation donated money to the National Institutes of Health to sequence the gene associated with a rare disease affecting the three grandchildren of Parseghian, the famed Notre Dame football coach. At the time, only a handful of labs in the world were working on Niemann Pick C, a neurodegenerative disorder known to strike one in 150,000 people.

Researchers knew the *NPC1* gene was behind most cases of NPC, and the disease was characterized by an accumulation of cholesterol in the lysosomes. But they were unsure of the location of the gene and how the protein functioned. Through integrated human-mouse positional cloning, researchers were able to narrow in on the *NPC1* gene.

After plugging the DNA sequence of the *NPC1* gene into the National Center for Biotechnology Information (NCBI) database, Jill Morris, a postdoc at NIH, remembers the excitement found within the 30-page print-out – the protein shared homology with 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase and sterol-regulatory element binding protein (SRP) cleavage-activating protein (SCAP), two proteins known to be involved in moving cholesterol in the cell, and PATCHED, a protein well-studied for its role in Hedgehog signaling in *Drosophila* (*Science*, 277:228–31, 1997).

Researchers immediately recognized the significance of the connection between NPC1 and other more well-studied proteins, says Bill Pavan, a molecular biologist who was part of the team and now works with animal models of skin disease at the NIH.

"By having links between this rare, understudied disease and these other areas of active research, I was excited to see we could attract additional researchers ... to help understand how mutation of the protein is involved in Niemann Pick C disease," Pavan says. Today, the Ara Parseghian Foundation funds 26 research projects into NPC and other cholesterol-related diseases, including cardiovascular disease and Alzheimer's disease (see feature on p. 32). ■